
TECHNIQUE OF ORGANIC CHEMISTRY
ARNOLD WEISSBERGER, *Editor*

Volume I

PHYSICAL METHODS OF ORGANIC CHEMISTRY

Second Completely Revised and Augmented Edition

Part Two

TECHNIQUE OF ORGANIC CHEMISTRY

ARNOLD WEISSBERGER, *Editor*

- Volume I:* Physical Methods of Organic Chemistry
Parts I and II
Second Edition
- Volume II:* Catalytic, Photochemical, and Electrolytic
Reactions
- Volume III:* Heating and Cooling; Mixing; Centrifug-
ing; Extraction and Distribution; Dialy-
sis and Electrodialysis; Crystallization and
Recrystallization; Filtration; Evaporat-
ing and Drying
- Volume IV:* Distillation
- Volume V:* Adsorption
- Volume VI:* Micro and Semimicro Methods
- Volume VII:* Organic Solvents
Second Edition



TECHNIQUE OF ORGANIC CHEMISTRY

Volume I—Part II

PHYSICAL METHODS of Organic Chemistry

Second Completely Revised and Augmented Edition

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ARNOLD WEISSBERGER

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PART TWO

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TECHNIQUE OF ORGANIC CHEMISTRY

INTRODUCTION

Organic chemistry, from its very beginning, has used specific tools and techniques for the synthesis, isolation, and purification of compounds, and physical methods for the determination of their properties. Much of the success of the organic chemist depends upon a wise selection and a skillful application of these methods, tools, and techniques, which, with the progress of the science, have become numerous and often intricate.

The present series is devoted to a comprehensive presentation of the techniques which are used in the organic laboratory and which are available for the investigation of organic compounds. The authors give the theoretical background for an understanding of the various methods and operations and describe the techniques and tools, their modifications, their merits and limitations, and their handling. It is hoped that the series will contribute to a better understanding and a more rational and effective application of the respective techniques. Reference is made to some investigations in the field of chemical engineering, so that the results may be of assistance in the laboratory and help the laboratory chemist to understand the problems which arise when his work is stepped up to a larger scale.

The field is broad and some of it is difficult to survey. Authors and editor hope that the volumes will be found useful and that many of the readers will let them have the benefit of their criticism and of suggestions for improvements.

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TECHNIQUE OF ORGANIC CHEMISTRY

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Volume VII. Organic Solvents (Second Edition). A. Weissberger, E. S. Proskauer, and J. A. Riddick

TECHNIQUE OF ORGANIC CHEMISTRY

Volume I

PHYSICAL METHODS OF ORGANIC CHEMISTRY

PREFACE to the First Edition

In recent years, the science of physics has become increasingly important to the organic chemist. Physics has given much greater precision to the concepts of atoms, bonds, and structural formulas, and it has made possible the development of new, and the improvement of older, methods for the examination of chemical systems. With the increasing number and complexity of physical methods for the treatment of organic chemical problems there has resulted a specialization of research workers in the methods which they employ, and the selection of a research problem is frequently governed more by the physical method to be used than by the chemical nature of the problem. Some workers have made themselves familiar with several methods in order to deal with their individual problems. In other cases, however, physical methods have been used without adequate preparation.

The chemist, in order to acquaint himself with a certain physical method, has in the past been compelled to search through periodicals and specialized books. The present work has been compiled with the hope of relieving him of much of this burden. It has been the object of the authors to provide a description of tested methods, the theoretical background for understanding and handling them, and the information necessary for a critical evaluation of the experimental results.

Because of the diversity of the methods discussed, no attempt has been made to secure a uniformity of presentation which might have been desirable for formal reasons. In some chapters a discussion of theory was unnecessary, in some a relatively brief theoretical treatment sufficed, and in other chapters a rather complete exposition of the theory appeared necessary. Some methods have been treated in monographs, while for others no comprehensive modern presentation is available. Therefore, a rather severe selection and delimitation of material was exercised in some chapters and a more complete treatment given in others.

The book is also calculated to appeal to the student who seeks to increase his understanding of the methods described, although he may not practice

them himself. For him, chapters like those on x-ray and electron diffraction should be adequate, but the practical application of these techniques will require the use of the supplemental literature to which reference is made.

The authors and editor will welcome any suggestions for improvements. The editor wishes to express his sincere thanks to the authors whose labor, often under difficult circumstances due to the war, made possible this treatise. He also acknowledges gratefully the assistance of his colleagues in reading the manuscripts, particularly of Drs. L. G. S. Brooker, I. Fankuchen, M. L. Huggins, E. E. Jelley, C. J. Kibler, E. Loewenstein, J. E. LuValle, J. Spence, J. Russell, C. V. Wilson and Messrs. R. H. Wagner and K. F. Weaver, and the help of Misses G. Das and A. M. Hyman in the clerical work.

The publishers and their staff deserve special recognition and thanks for their eager and understanding cooperation.

A. W.

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P R E F A C E

to the Second Edition

The purpose of this treatise is stated in the preface to the first edition. The second edition has been enlarged by five new chapters (I, II, V, XIII, XXVI), by new sections in chapters XV and XXII, and by a substantial expansion of many of the other chapters; some of them have been entirely rewritten (VIII, XI, XII, XXIV, XXX, XXXI), and others have been thoroughly revised.

New chapters V, XIII, and XXVI deal with the determination and evaluation of Vapor Pressure, with measurements made with the Ultracentrifuge, and with Electrophoresis. In the additions to chapters XV and XXII are presented Electron Microscopy, Nephelometry, and the determination and evaluation of Light Scattering, respectively. Temperature Measurement and Temperature Control are important for so many of the methods that it seemed advisable to treat them separately in chapters I and II, to which reference is made in the other chapters. Likewise, a section on Light

Sources has been added in chapter XXI. However, special applications of these general subjects are presented in the chapters which deal with the respective problems.

We hope that the new edition provides a more homogeneous and complete presentation of the field than the first edition. The title, *Physical Methods of Organic Chemistry*, has been called too narrow. "Organic Chemistry" distinguishes the methods described from those physicochemical methods which, though essential in other fields, are less important for, or not applicable to, organic chemistry—for example, methods employing very high temperatures. We do not, of course, suggest that the methods described are applicable to organic problems only. Though it is gratifying that workers in other fields have found the book useful, it is our chief object to provide information on the physical methods used by chemists, physicochemists, physicists, biologists, and other research workers in dealing with organic chemical problems.

In the volume at hand are described the *determination and evaluation of physical properties* of organic compounds. Other volumes of the present series deal with techniques used in the *preparation, isolation, and purification* of organic compounds.

I acknowledge with gratitude the valuable suggestions made in reviews and in letters by Drs. H. G. Cassidy, N. D. Cheronis, S. Glasstone, and W. Swietoslawski and the assistance of Drs. L. C. Craig, E. M. Crane, A. L. Geddes, L. Hess, K. C. D. Hickman, M. L. Huggins, W. J. Knox, V. K. LaMer, L. G. Longworth, R. P. Loveland, J. E. LuValle, H. Mark, D. R. Morey, W. R. Ruby, D. R. Simonsen, D. W. Stewart, J. M. Sturtevant, R. S. Tipson, G. W. Thomson, and L. H. Weissberger and Messrs. A. Ballard, R. L. Bent, C. A. Morrison, and H. Shapiro in reading the manuscripts. Miss K. Goepf assisted the authors in preparing chapters XI and XII. Dr. S. Frank prepared the revised index. I am grateful for their efforts and for the splendid and understanding cooperation of the publishers in the planning and the preparation of the new edition.

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TECHNIQUE OF ORGANIC CHEMISTRY
Volume I

PHYSICAL METHODS OF ORGANIC CHEMISTRY

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X-RAY DIFFRACTION

I. FANKUCHEN, *Polytechnic Institute of Brooklyn*

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I. INTRODUCTION

X-ray diffraction has long been used as a tool in chemistry. In 1916, Debye and Scherrer¹ and, in 1919, Hull² described the powder method of x-ray diffraction. Subsequently, this method became an important one in chemistry almost to the exclusion, unfortunately, of other x-ray diffraction techniques. It is easy to understand this trend, for the powder technique is simple and does not require elaborate apparatus, and the interpretation of the x-ray diagrams is a comparatively simple procedure. However, the powder method is limited in scope: in most cases it can only be used for identifying materials and this, for chemists, is now its chief use. In the inorganic field, one generally does not want more from x-ray diffraction; but the organic chemist, who often works with materials of unknown

¹ P. Debye and P. Scherrer, *Physik. Z.*, **17**, 277 (1916)

² A. W. Hull, *J. Am. Chem. Soc.*, **41**, 1168 (1919).

structure, would like an additional tool for working out these structures. Single-crystal x-ray studies can often give useful information in such cases, information which cannot be obtained by the powder method. The interpretation of single-crystal x-ray diagrams is not, however, an easy matter. Unless one uses reciprocal-lattice ideas—and these do not appear to be well enough known—the interpretation of single-crystal diffraction films becomes an exceedingly laborious task. Because, for all types of x-ray diagrams, the use of the reciprocal lattice simplifies the interpretation, what may perhaps appear to be unnecessary emphasis will be given to its discussion. The discussions throughout this chapter will be limited strictly to those ideas and facts essential to the organic chemist wishing to work with x-ray diffraction methods.

II. X-RAYS

1. Production and Equipment

X-rays are produced when electrons moving with sufficiently high velocities are suddenly stopped by impact with a target. The essential parts of all x-ray apparatus are similar. A source of electrons—usually a hot filament—a high tension source for accelerating the electrons, and a solid obstacle, the target, must be provided. There are many purely technological details. For instance, electrons even of very high energies are easily absorbed, and so the target and electron source must be maintained in a vacuum; targets get hot from the electron bombardment, and so must be cooled; and the x-rays usually used for x-ray diffraction work are also easily absorbed, so that thin windows of low absorbing material, Lindemann glass, or beryllium, must be provided in the walls of the tube to permit the emergence of the x-ray beam.

For most work the organic chemist may wish to do, the sealed-off x-ray tubes now commercially available (General Electric, Machlett, and Philips for example) will do very well. These are available in a large variety of types and target materials. The copper target tube is the best all-round tube although special conditions may dictate the choice of some other target material. All commercial equipment uses transformers as the source of the high voltage. For many reasons, it is desirable that some rectification, either half-wave or full wave, be used.

Because the design of auxiliary equipment, cameras, monochromators, etc., is still in a fluid state, it is highly desirable that any equipment obtained should have a flat working surface which can be cleared of encumbrances so special apparatus can be used without extensive modifications.

At this time, at least three manufacturers offer complete x-ray diffraction equipment suitable for routine x-ray diffraction work: *North American*

Philips, New York; *General Electric X-Ray Corporation*, Milwaukee; *Picker X-Ray Corporation*, New York. Because most industrial laboratories employ the powder method (p. 1087), these manufacturers emphasize this particular type of diffraction work in the design of the apparatus. In all three, a wide variety of target materials is available, and arrangements can be made for the use of single-crystal apparatus. Each commercial apparatus, at present, has its advantages and disadvantages, although the pressure of competition will undoubtedly dictate such modifications that the differences should soon not be very great. The Philips unit, for example, has four windows of thin mica, while the other outfits have only two of beryllium.

2. Properties

X-rays are electromagnetic waves (like light) of very short wave length. It is convenient, in x-ray work, to use as a unit of length the angstrom unit (\AA), equivalent to 10^{-8} centimeter. X-rays cover the region from 0.01 to 1000 \AA ., overlapping on the short wave length side the softer gamma rays and, on the long wave length side, the extreme ultraviolet. For diffraction work, wave lengths between 0.7 and 2 \AA . are generally used.

The spectral distribution of the x-rays emitted by targets under varying conditions can be studied by absorption measurements, crystal diffraction, and ruled-grating techniques. It can be shown that the spectrum is usually composed of a background of continuous (white) radiation upon which is superimposed a more or less complex line pattern called the characteristic line spectrum. The white radiation cuts off sharply at a minimum wave length which depends only on the peak voltage applied to the x-ray tube. The relation is:

$$\lambda_{\min.} = 12,350/V \quad (1)$$

where $\lambda_{\min.}$ is the minimum wave length in angstrom units and V is the peak potential in volts. The shape and intensity of the white radiation spectrum depend, however, on the target material and on the shape of the applied voltage wave.

The characteristic line spectrum is a characteristic of the target material (hence the name). The wave lengths of the lines are independent of the voltages used, but the appearance or nonappearance of the various lines does depend on the applied voltage. The characteristic lines originate in electron jumps to excited electron shells within the target atoms, and so the various sets of lines (K, L, M, etc., in order of increasing wave length, *i. e.*, decreasing energy) can be produced only when the bombarding electrons have sufficient energy to ionize the respective shells concerned

(K, L, M, etc.). For almost all diffraction work, it is the characteristic K lines that are used, specifically the $K\alpha$ doublet. The $K\beta$ component is usually removed by filtering (see page 1077). For maximum contrast of K lines to background, the peak potential applied to the x-ray tube should be from 3.5 to 4 times the excitation potential of the K shell. The excitation potential is obtained by substituting the wave length of the K edge (Table I) in equation (1).

TABLE I
WAVE LENGTHS OF K LINES AND EDGES OF SOME IMPORTANT TARGET
AND FILTER MATERIALS

Target material	Atomic number	Wave length in \AA X units of			
		$K\alpha_1$ line	$K\alpha_2$ line	$K\beta$	K absorption edge
Ti	22	2.7468	2.7432	2.5090	2.4912
V	23	2.5021	2.4983	2.2797	2.2630
Cr	24	2.2889	2.2850	2.0806	2.0659
Mn	25	2.1015	2.0975	1.9062	1.8916
Fe	26	1.9360	1.9321	1.7530	1.7394
Co	27	1.7892	1.7853	1.6174	1.6040
Ni	28	1.6583	1.6545	1.4970	1.4839
Cu	29	1.5412	1.5374	1.3893	1.3774
Zn	30	1.4360	1.4322	1.2925	1.2805
Zr	40	0.7885	0.7843	0.7003	0.6874
Mo	42	0.7128	0.7078	0.6310	0.6185

The absorption of x-rays in traversing matter is given by:

$$I = I_0 e^{-\mu t} \quad (2)$$

where I_0 is the intensity of the beam incident upon the specimen, I is the intensity after passing through a thickness t and μ is the linear absorption coefficient. This equation can be obtained by a simple integration, the basic assumption being that, for very thin specimens, the absorption is proportional to the thickness. The linear absorption coefficient is, however, dependent on the state of the absorber; it is therefore often convenient to use instead μ_m , the mass absorption coefficient. This coefficient, $\mu_m = \mu/\rho$ where ρ is the density, is independent of the physical or chemical state of the absorber and is, for any given element, a function of the wave length, λ , of the radiation being absorbed. X-rays by absorption can also produce ionization of the various electron levels of the absorbing atom. If one studies the mass absorption coefficient of an atom as a function of wave length, one then observes that, with increasing wave length, there is an increase of absorption coefficient up to the wave length corresponding to that of the K absorption edge, then a sudden drop and a gradual increase to the succeeding L and M edges which, for our diffraction purposes, are of little interest. The K edge, however, is widely used to eliminate the $K\beta$

line from the radiation emitted by the tube. One chooses as a filter a material whose K edge lies between the wave lengths of the $K\alpha$ and $K\beta$ lines. The $K\alpha$ will then pass through with little absorption while the $K\beta$ will be highly absorbed. Thus, for copper targets nickel filters are used. Nickel foil 0.0007 inch thick will absorb only 50% of the $K\alpha$ radiation but will reduce the $K\beta$ component to $1/35$ of its original strength. Inasmuch as, in

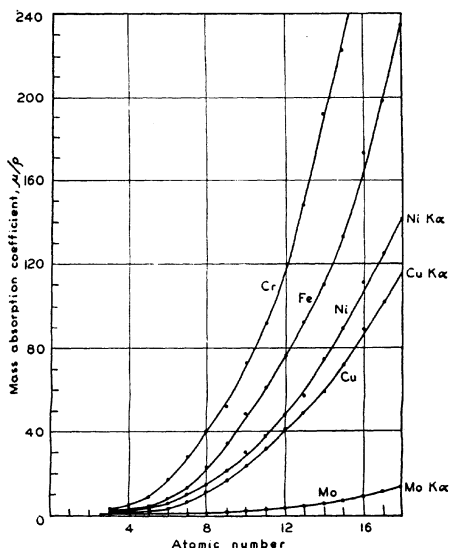


Fig. 1.—Mass absorption coefficients of the low atomic number elements.

the original beam, the ratio of $K\alpha$ to $K\beta$ is about 6 to 1, the ratio in the filtered beam will be 250 to 1.

Sometimes the presence of absorption edges dictates the choice of radiation. A material rich in iron, for instance, should not be studied with copper radiation, as the $K\alpha$ line of copper will excite the K level of the iron (see Table I), producing a heavy background of fluorescent radiation.

A knowledge of the absorption coefficients is necessary for determining the best specimen size. Thus, in the usual powder method in which a cylindrical specimen is used with a beam of diameter greater than the specimen cross section, the optimum specimen diameter is $2/\mu$ centimeter,

where μ is the *linear* absorption coefficient. For a full discussion of optimum specimen size see Buerger.³

Figure 1 is a graph* giving the mass absorption coefficients as a function of atomic number. To find the mass absorption coefficient of a material of composition A B C . . . Z, one uses the equation:

$$\mu_{ABC\dots Z} = \frac{A\mu_A + B\mu_B + \dots + Z\mu_Z}{A + B + C + \dots + Z} \quad (3)$$

where $\mu_{ABC\dots Z}$ is the *mass* absorption coefficient of the compound, A, B, C . . . Z are the weight percentages (or atomic weights) of the constituent atoms present, $A + B + C + \dots + Z = 100\%$ (or the molecular weight), and $\mu_A, \mu_B, \mu_C, \dots, \mu_Z$ are the mass absorption coefficients of the constituent atoms.

For a thorough discussion of the physics of x-rays, see Compton and Allison.⁴ Suitable textbooks⁵ should be referred to for technical data on x-ray production and apparatus.

III. ELEMENTS OF CRYSTALLOGRAPHY

A crystal is a regular three-dimensional assemblage of some fundamental repeat unit. The crystal form can be correlated to the form of the repeat unit (the unit cell), and it is the function of the x-ray crystallographer to determine the size and shape of the unit cell and also, when possible, the atomic distribution within the unit cell. The organic chemist is very much interested in the structure of the molecules he deals with but not in the appearance of the crystals save as they serve to characterize the substances. It would appear that the chemist would be satisfied with nothing less than a *complete* structure determination, since this alone gives him a full knowledge of the structure of the molecules which make up the crystal. It is, however, difficult, and in most cases at the present time impossible, to work out the complete structures of crystals of complex organic molecules. However, our inability to solve the problem completely does not prevent the x-ray approach from giving valuable information about the substances investigated.

Particularly for single-crystal studies, a knowledge of crystallographic

* I am indebted to F. G. Chesley for the suggestion of plotting absorption coefficient as a function of atomic number. The data for figure 1 are from W. L. Bragg, *The Crystalline State*, Macmillan, New York, 1934, Appendix III.

³ M. J. Buerger, *X-Ray Crystallography*. Wiley, New York, 1942, p. 179.

⁴ A. H. Compton and S. K. Allison, *X-Rays in Theory and Experiment*. Macmillan, New York, 1935.

⁵ G. L. Clark, *Applied X-Rays*, 3rd ed., McGraw-Hill, New York, 1940. C. S. Barrett, *Structure of Metals*, McGraw-Hill, New York, 1943.

nomenclature is essential. Much of this is given by Peacock in Chapter 16 (page 983). For a more extended treatment from the point of view of the x-ray crystallographer, see Buerger.⁶ Peacock deals with the crystal lattice, or translation lattice, which is defined by a unit cell with edges a , b , c , and angles α , β , γ ; the Miller indices (hkl) used in denoting lattice planes and x-ray reflections; the 14 lattice types, including the seven primitive types (P) and the seven centered types (I , C , F); the grouping of the lattice types into seven or six systems; the elements of symmetry which do not involve translations, namely the rotation axes (1), 2, 3, 4, 6, and the inversion axes, or their equivalents, $\bar{1}$ (center of symmetry), $\bar{2}$ (mirror— m), $\bar{3}$, $\bar{4}$, $\bar{6}$ ($3/m$); and the various combinations of these symmetry elements to give the 32 crystal classes or point groups. The elements of symmetry involving translations, and their combination in the 32 classes and the 14 lattices to give the 230 space-groups or ultimate symmetry types, are only mentioned; and therefore a short explanation of these symmetry elements and groups is given here to complete the outline of crystallographic nomenclature.

The elements of symmetry which involve translation, in addition to rotation or reflection, are the screw axes and the glide planes, respectively. An n_p screw axis is an n -fold rotation axis with a translation of p/n of the unit length in the direction of the axis; thus a 4_1 screw is a symmetry element in which a rotation of $2\pi/4$ is combined with a translation of one-fourth of the unit length. Similarly the translations accompanying a glide plane are always parallel to the plane and are a simple fraction of some cell dimension. Thus, an a -glide plane translates $a/2$, b translates $b/2$, and c translates $c/2$; an n -glide plane translates one-half the diagonal of the face to which it is parallel; and a d -glide plane has a translation of one-fourth of the face diagonal.

Various combinations of these symmetry elements are possible in the different crystal systems. Thus, the only symmetry element possible in the triclinic system is a center of symmetry, and 3-fold axes are not found in the monoclinic, orthorhombic, and tetragonal systems. A possible arrangement of symmetry elements placed at the lattice points of a space-lattice is called a space-group. A space-group is *not* a crystal structure nor an array of atoms or molecules. It has been shown that there are 230 possible space-groups; all have been listed and described in detail.⁶⁻⁹

⁶ M. J. Buerger, *X-Ray Crystallography*. Wiley, New York, 1942.

⁷ *International Tables for the Determination of Crystal Structures*. Bell, London, 1935. J. W. Edwards, Ann Arbor, 1944.

⁸ K. Lonsdale, *Structure Factor Tables*. Bell, London, 1936.

⁹ R. W. G. Wyckoff, *The Analytical Expression of the Results of the Theory of Space Groups*. Carnegie Institution of Washington, Washington, D. C., 1930.

The modern nomenclature for the space-groups (Hermann-Mauguin) is one from which the space-group can be constructed from the symbol. The first part of the symbol is a letter which refers to the space-lattice: P is a primitive cell, A , B , C are face-centered on the a , b , c faces, respectively, F is all face centered, I is body-centered, H is hexagonal, and R is rhombohedral. The next symbol, if a number, refers to the principal axis, 2, 3, 4, or 6. The symbol for a reflection plane is m (mirror), and $2/m$, $3/m$, etc., stand for rotation axes with perpendicular mirror planes. If the axes are screw axes or the planes are glide planes the corresponding symbols are used (see above). Symbols for additional axes or planes then follow. For a full discussion, see Bragg¹⁰ or Buerger.¹¹

If a model of a space-lattice is slowly turned, it can be seen that the lattice points may be considered as lying in various sets of parallel planes. Peacock (page 983) discusses in detail the indexing of these planes. Any such set can be identified by the three Miller indices (hkl). Consider any such set of parallel planes. They cut the a , b , and c axes into different numbers of parts. If " a " is divided into " h " parts, " b " into " k ," and " c " into " l " parts, then hkl are the Miller indices of that set of planes. If a plane (set of planes) is parallel to an axis, then the corresponding index is zero. It will be observed that h , k , and l as defined have no common factor. In discussing x-ray reflection by crystals, we shall have occasion to use sets of indices which have a common factor. This common factor is the order of the reflection. It is merely more convenient to speak of the (3, 12, 15) reflection than to say "third order of the (1, 4, 5) set of planes."

IV. REFLECTION OF X-RAYS BY CRYSTALS

1. Characteristics of Patterns

X-rays are used to study crystals because the pattern of scattered x-rays somehow reflects the order and regularity of the atomic arrangement within the specimen. All x-ray diffraction apparatus have certain characteristics in common: a system of slits or pinholes which defines the x-ray beam, a means for holding and moving the specimen in the x-ray beam, and a radiation-sensitive device—usually a photographic film—to record the scattered radiation. Photographs will vary widely in their appearance depending on the specimen character. Four representative types can be seen in figure 2. When the specimen has no crystalline order whatsoever (a piece of glass or carbon black), one nevertheless obtains a diagram (Fig. 2A) with certain distinctive characteristics, namely, there are one or more

¹⁰ W. L. Bragg, *The Crystalline State*. Macmillan, New York, 1934.

¹¹ M. J. Buerger, *op. cit.*, Chapter IV and Table 7.

halos. These arise because atoms have finite sizes and indicate that two atoms cannot approach closer than the sum of their radii. From a study of such diagrams something may be determined about the sizes of the atoms and their environments; for instance, x-ray diagrams of silica glass indicate that silicon-oxygen tetrahedra of a definite size are present.

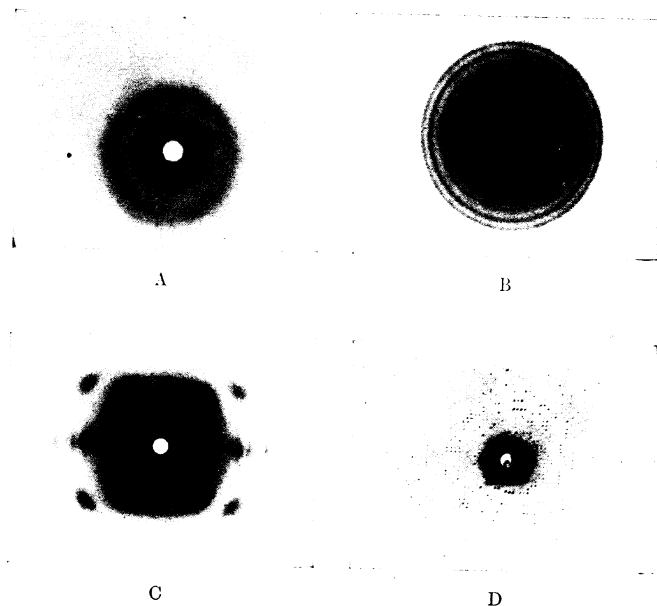


Fig. 2.—Typical x-ray diagrams.

- A—Amorphous diagram
- B—Crystalline powder diagram (puffed starch)
- C—Fiber diagram (Saran)
- D—Single crystal oscillation diagram (γ -chymotrypsin)

When the specimen consists of powdered crystals, one again obtains a series of rings (Fig. 2B); they are now sharp, not diffuse. From the locations of the rings, it is sometimes (not always—this is one of the weaknesses of the powder method) possible to deduce the size and shape of the unit cell. Because the material is crystalline, the lines are sharp in contrast to the diffuse halos of the first example. However, both specimens have no

specific orientation, as is shown by the continuous even rings obtained, diffuse for the amorphous and sharp for the crystalline case.

In figure 2C, the specimen is a typical fiber with not too perfect crystallinity or orientation. Here, instead of rings, there are spots—broad because the crystalline arrangement is not very good, and somewhat elongated into arcs because of imperfect orientation. The spots fall on “layer” lines which are perpendicular to the fiber axis. From the layer-line separation, the fiber period can be computed unequivocally. Finally, a good single crystal (when oscillated) gives the pattern of figure 2D, a pattern of sharp spots arranged on layer lines and row lines. In this particular case, the specimen, γ -chymotrypsin, is tetragonal, and the square character of the spot pattern shows that the specimen was oriented with one of the equal axes vertical and that during oscillation the x-ray beam was directed approximately along the tetragonal axis.

2. Bragg's Law

The reflection of x-rays by crystals is really an interference phenomenon. Considered fundamentally, every atom of the crystal bathed in the beam of x-rays is a secondary radiating source in which the wave length and phase are maintained unchanged. Laue has shown that, for a three-dimensional regular array, these secondary waves interfere with one another in such a way that, except for certain calculable directions, destructive interference occurs. In the special directions, however, there is constructive interference and strong x-ray beams can be observed. But this treatment is too complicated; and Bragg's great contribution is a much simpler treatment of the scattering phenomenon. First, he shows that scattering centers arranged in a plane act like a mirror to x-rays incident on them, *i. e.*, constructive interference occurs for the direction of specular reflection. Through the points of a space-lattice, an infinite number of sets of parallel planes can be passed. Consider one such set of Miller indices (hkl). Each plane of this set reflects part of the incident beam specularly. Let the x-rays be monochromatic of wave length λ . For an arbitrary glancing angle, θ , the reflections from successive planes are out of phase with one another and destructive interference occurs. However, by varying θ , a set of values for θ can be found so that the path difference between x-rays reflected by successive planes will be an integral number of wave lengths, n , and then constructive interference occurs. In figure 3, the horizontal lines are the traces of two successive planes of the set. The spacing of these planes, d , is the perpendicular distance between them. The value of d can always be computed from the dimensions of the unit cell and the value of (hkl).¹²

¹² M. J. Buerger, *X-Ray Crystallography*. Wiley, New York, 1942, p. 103.

The path difference is $BC + CD$ and for constructive interference this must equal $n\lambda$. But $BC + CD$ is equal to $2d_{hkl} \sin \theta$. Putting these facts together we obtain Bragg's law:

$$n\lambda = 2d_{hkl} \sin \theta$$

It can be seen that, unlike the case of diffraction of light by a ruled grating, this phenomenon is critical; constructive interference (reflection) occurs only at certain critical values, θ_n , the Bragg angles. When reflection does occur it is stated that the (hkl) planes are reflecting in the n th order or, more simply, it is called the $(nh\ nk\ nl)$ reflection.

Consider a crystal with a given unit cell containing p atoms per unit cell. Taking each of the p atoms in succession as origin of a space-lattice, we can imagine the crystal as composed of p identical (as far as size and shape are concerned) but interpenetrating lattices, their displacements relative to one another being decided merely by the distribution of the p atoms within the unit cell. All these lattices will have identical Bragg angles, but the reflections from the p -independent lattices will interfere with one another. This interference will determine the intensity of the reflection h, k, l ; but the Bragg angle will be independent of the atomic distribution *within* the cell, depending only on the external cell geometry.

When a nonprimitive unit cell is chosen, systematic absences of reflections will be observed. In a body-centered cell, only those reflections will occur for which $h + k + l$ is even; in an A face-centered lattice, $k + l$ must be even (in a B -lattice $h + l$, in a C , $h + k$); while in an F -type lattice, h, k , and l must be all odd or all even.

Screw axes and glide planes also result in the absence of certain types of reflections; thus, a b (or c) glide plane perpendicular to the " a " axis causes $(0kl)$ reflections to vanish for k (or l) odd, an a (or c) glide plane perpendicular to the b axis results in the extinction of $(h0l)$ reflections for h (or l) odd, etc. Similarly, a screw axis parallel to the a or b or c axis affects the $(h00)$ or $(0k0)$ or $(00l)$ reflections, respectively. A 2_1 screw axis permits only even orders to occur, a 3_1 screw axis only those divisible by 3, etc. For a full treatment, see Bragg¹³ and Buerger.¹⁴ It is by a study of external sym-

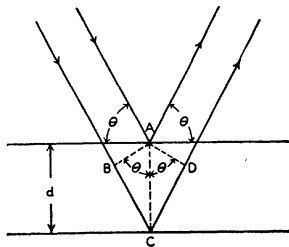


Fig. 3.—Bragg's law.

¹³ W. L. Bragg, *The Crystalline State*. Macmillan, New York, 1934, Chapter VI.

¹⁴ M. J. Buerger, *op. cit.*, p. 505.

metry, optics (polarizing microscope studies), and systematic extinctions that the space-lattice type and finally the space-group are determined.

3. Analysis of Patterns

One can now see that the analysis of x-ray diagrams falls into three parts, a geometrical analysis, an intensity analysis, and lastly what may be called a study of the quality of the pattern.

The complete geometrical analysis is straightforward for single-crystal x-ray diagrams, but is not always possible for powder diagrams. One measures the *locations* of x-ray reflections and computes from these measurements the size and shape of a unit cell. From a study, then, of the intensities of the various reflections, one *attempts* to determine the atomic distribution within the unit cell. An ambiguity enters here, however, which will be discussed later (page 1104), hence the use of the word "attempts." Finally, one can look at the diagram and say the lines are broad (or perhaps sharp) and the crystalline arrangement is therefore not too good (or perhaps perfect); the spots are drawn out, the fiber (if it is a fiber) is therefore not well oriented, etc. One can even measure the breadth of the x-ray reflections and the angular arc over which the spots are elongated and make quantitative deductions as to the perfection of the crystalline order and orientation.

V. RECIPROCAL LATTICE THEORY

For every possible set of Bragg planes of indices (hkl) —using (hkl) hereafter in the broader sense, *i. e.*, (hkl) may have a common factor—there is a reflection which may be characterized by the same triad (hkl) , *i. e.*, there is a 1:1 correspondence between sets of Bragg planes and spots on a photographic film. The reciprocal lattice bears a similar relation to the set of possible reflecting planes; each *point* of the reciprocal lattice corresponds to a set of reflecting planes in the real lattice (and vice versa). Therefore, there is a 1:1 correspondence between points of the reciprocal lattice and reflections on the photographic film. Ewald, and then Bernal,¹⁵ made use of this concept to obtain a very elegant geometrical interpretation of Bragg's law, an interpretation in which reflection from a plane in real space is replaced by the drawing of a straight line to a reciprocal lattice point. It will now be shown that this mathematical inversion makes possible the indexing of diffraction films in an easy, and indeed natural, way.

To construct the lattice reciprocal to a given space-lattice, an arbitrary origin is first chosen. From this origin, normals are drawn to the different

¹⁵ J. D. Bernal, *Proc. Roy. Soc. London*, **A113**, 117 (1927).

sets of Bragg planes of indices (hkl) . Point hkl in reciprocal space corresponding to planes (hkl) lies on the normal to planes (hkl) at distance d^* from the origin equal to K^2/d_{hkl} where K is an arbitrary constant and d_{hkl} is the spacing of the set of planes. It is most convenient to take $K^2 = \lambda$, the x-ray wave length (following Bernal¹⁵), so:

$$d_{hkl}^* = \lambda/d_{hkl} \quad (4)$$

The unit cell of a space-lattice is defined by $a, b, c, \alpha, \beta, \gamma$, or, what is equivalent, by the three pairs of planes which form the sides of the unit cell. Their Miller indices are $(100), (010), (001)$. Now consider the arbitrarily chosen origin of the reciprocal lattice and the three points reciprocal to these three planes. They will be labeled (have the coordinates) $100, 010, 001$, respectively. These four points define a lattice of reciprocal points: thus it can be shown that the point of coordinates $1, 2, 3$ in reciprocal space will be reciprocal to the set of planes of Miller indices (123) and more generally the point hkl in the reciprocal lattice will be reciprocal to the (hkl) set of planes in the real lattice.¹⁶

To demonstrate the usefulness of the reciprocal lattice, we use it now to compute the formula for d_{hkl} for a cubic crystal. The reciprocal lattice will also be cubic of edge $a^* = \lambda/a$. Point hkl in the reciprocal lattice will be at a distance $d^{*2} = h^2a^{*2} + k^2a^{*2} + l^2a^{*2}$ from the origin. By definition:

$$d_{hkl} = \frac{\lambda}{d_{hkl}^*} = \frac{\lambda}{a^* \sqrt{h^2 + k^2 + l^2}} = \frac{a}{\sqrt{h^2 + k^2 + l^2}} \quad (5)'$$

This computation is, of course, very simple for the cubic case even without using the reciprocal lattice. For noncubic cases, however, the analogous computation is much simpler by reciprocal-lattice methods.

With the introduction of the sphere of reflection, some of the potentialities of the reciprocal lattice become apparent. In figure 4, let AB be the direction of a beam of x-rays of wave length, λ , incident upon a crystal at C . With C as a center, construct a sphere of unit radius to any convenient scale, say five centimeters equals unity. Line AB emerges from this sphere at O . With O as an origin, construct the reciprocal lattice for the crystal at C using the same scale as for the sphere. If the crystal at C is rotated about an axis through C , then the reciprocal lattice will rotate in the same way about a corresponding axis through O and in general any rotations about C by the crystal will be followed by corresponding rotations of the reciprocal lattice about O . In general, for an arbitrary orientation of the crystal, no point of the reciprocal lattice will lie on the surface of the unit

¹⁶ M. J. Buerger, *X-Ray Crystallography*. Wiley, New York, 1942, Chapter VI.

sphere. The crystal is rotated so that a point, P_{hkl}^* , of the reciprocal lattice lies on the surface of the sphere. We now assert that the set of Bragg planes of indices (hkl) are in a reflecting position, *i. e.*, obey Bragg's law, and that moreover the direction of the reflected ray is from crystal C to point P_{hkl}^* . The proof is obvious. Since P_{hkl}^* is the reciprocal point corresponding to planes (hkl) , line OP is normal to planes (hkl) . The plane, normal to the paper, of which CD is the trace is drawn parallel to planes (hkl) , *i. e.*, perpendicular to OP . It bisects angle PCO and line OP . Since OC is unity, OP is $2 \sin \alpha$. But OP is also λ/d_{hkl} (Eq. 4). Equating, we obtain $\lambda = 2d_{hkl} \sin \alpha$, *i. e.*, α is the Bragg angle, θ . Also, one sees from the diagram that CP is the direction of the reflected ray, making angle of incidence equal to angle of reflection.

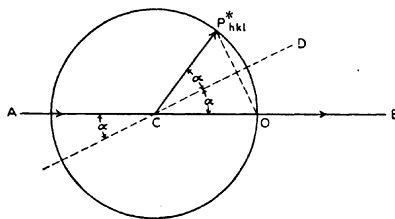


Fig. 4.—The sphere of reflection.

This proof for one point, hkl , of the reciprocal lattice holds, of course, for all points of the reciprocal lattice. One can now see clearly the critical character of Bragg's law. Because the reciprocal lattice is a discrete array of points, there will, for an arbitrary orientation of the crystal relative to a monochromatic x-ray beam, be no reflected rays. As the crystal is rotated, the points of the reciprocal lattice pass through the surface of the sphere. At the moment of transit, the corresponding planes will reflect and the directions of the reflected rays will be from point C to the reciprocal points at transit. One also sees clearly that points close together in reciprocal space will correspond to reflections close together on the film. In fact, for most techniques, the pattern one obtains is a distorted but recognizable projection of the reciprocal lattice. For points close to the origin, the distortion is slight. Thus, in figure 2D, the square character of the pattern is not very distorted and reflects the tetragonal reciprocal lattice. In fact, knowing that the crystal was tetragonal one could say merely by looking at the diagram that the oscillation was about the "a" axis and that the x-ray beam at one end of the oscillation was almost parallel to the tetragonal axis.

One can assign to the points of the reciprocal lattice weights which will

be proportional to the intensities of the x-ray reflections. This weighted reciprocal lattice will then contain all the information one can obtain by x-ray methods; and the intensity analysis is really the transformation of the weighted reciprocal lattice into an atomic distribution in real space. The transformation is not unique in this direction (the reverse transformation from real to reciprocal space is) and generally cannot be carried out unless sufficient additional non-x-ray information is available.

VI. METHODS AND APPARATUS FOR DIFFRACTION MEASUREMENTS

For the organic chemist, only those methods using monochromatic x-rays are of much interest. These methods use a polycrystalline specimen (the powder method), a fiber, or a single crystal (rotation, oscillation, and moving-film methods).

1. Powder Method

The powder method is very simple technically but gives the least amount of information. Its principal uses are for identification, precision determination of lattice constants, and particle size determination.

The specimen is usually prepared in the form of a cylinder. Depending on the material studied, it may be sealed in thin-walled glass capillaries, extruded in the form of a cylindrical rod, or caused to adhere to the outside of a glass fiber by using an appropriate adhesive (thin shellac, for example). Either a cylindrical camera with the rodlike specimen at the center or a flat film perpendicular to the x-ray beam is used. Means must be provided for accurately centering the specimen and for rotating it about its axis during the exposure. It is important that the specimen diameter should be close to the optimum value, $2/\mu$ centimeter. Appreciable departure from this optimum value results in longer exposures and unduly heavy backgrounds, and may even give misleading relative intensities. Fortunately for organic materials and the common radiations, this optimum value ranges conveniently from 0.5 to 1.0 millimeter, and therefore dilution with light material or other artifices is usually not necessary.

It is important that the specimen holder be so designed that the specimen can be properly adjusted in a convenient manner. Most commercial powder cameras have no provision for mechanically orienting the cylindrical specimen parallel to the axis of rotation. Such adjustment should be first made by mounting the specimen in Plasticine and then moulding the Plasticine until the parallelism is achieved. The specimen holder should have a slide mechanism for centering the specimen; this adjustment can be easily made once parallelism has been obtained. When a specimen is

properly adjusted, no transverse movement of the specimen should be noticed as it is rotated.

Several alternative methods of preparing cylindrical specimens have been mentioned. If thin-walled glass capillaries are used, they should be drawn from low-absorption (for x-rays) glass tubing, Pyrex, or Lindemann glass. Soda glass should definitely be avoided. The internal diameter of such capillaries should be between 0.3 and 0.8 millimeter and the wall thickness (for Pyrex) should be less than 0.05 millimeter. Filling such capillaries is sometimes a problem, as they should be so fine that any appreciable finger pressure will crush them. An easy method is sealing one end by means of a microflame. The open end is pushed into a small heap of the material to be examined and then held vertically upwards while the capillary is caused to vibrate by stroking it gently with a fine file. The vibrations will cause the powder to fall to the closed end. The process is repeated until enough specimen has accumulated at the bottom. It can be then compacted into place by a fine glass ramrod of appropriate diameter. If the powder consists of coarse crystals, it should be ground and screened through a fine wire mesh before insertion in the capillary. If this is not done, the resulting x-ray diagrams will consist of lines of a spotty—rather than uniform—character.

The glass of the capillaries of course gives some halos in the x-ray diagram and absorbs a large fraction of the useful $K\alpha$ radiation. It is, moreover, not too easy to draw out suitable capillaries. For these reasons, extruded specimens are being more widely used. The material to be studied is wet with a suitable binder such as thin collodion or shellac or even water, and then is packed into a short length of thick-walled glass capillary of the desired bore. A piston of piano wire can be used to compact the mass and finally to extrude it. The specimen will adhere to the piston end, which can then be used as the specimen support if a short enough piston has been used. Some precautions are necessary with this technique. The glass capillaries should be cut off squarely at the extrusion end or the specimen cylinder may curve as it emerges from the capillary. With different materials, varying amounts of binder will have to be used, and some experimentation will be necessary to determine the optimum conditions.

The powder method works despite the critical nature of Bragg's law because of the random orientation of the myriad of crystallites which make up the specimen. These will make every conceivable angle with the x-ray beam, and therefore will cause every possible reflection to occur. It is easier to see what happens if one thinks in terms of the reciprocal lattice. In figure 4, there is a single crystal at C and a discrete reciprocal lattice at O . Now if the crystal at C is replaced by a powder of randomly oriented crystallites, there will be centered at O a myriad of identical reciprocal lattices,

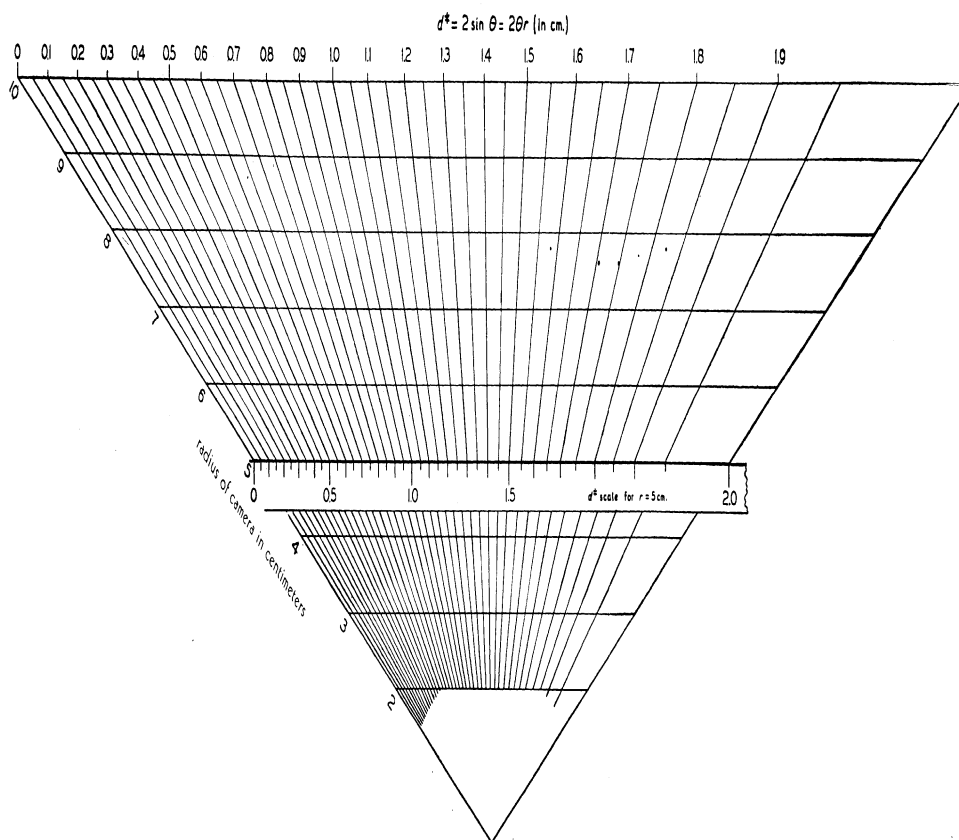


Fig. 5.— d^* ($2 \sin \theta$) scale for cylindrical powder cameras.

all oriented at random. Each point hkl at distance d_{hkl}^* from O is then replaced by a sphere of radius d_{hkl}^* with center at O . This set of concentric spheres intersects the unit sphere (the sphere of reflection) in a series of circles; and, following the previous derivation, there will be reflected rays from C to these circles. Thus the reflected rays will form a series of coaxial cones. If a film perpendicular to the x-ray beam is used to record the reflections, the result will be a series of concentric circles; a cylindrical film will intersect the cones in a set of more complicated curves.

Obviously, the dimensions of the rings (or curves) are related to the spacings of the planes responsible for the reflections. The half-angle of the cones (*i. e.*, the angle of deviation) is 2θ where $\lambda = 2d_{hkl} \sin \theta$.

For flat films, the diameter of the ring, d , is given by $d/s = 2 \tan 2\theta$ where s is the specimen-to-film distance. For cylindrical films, the corresponding distance, d , is given by $d/r = 4\theta$ in radians, where r is the camera radius.

One can measure d very accurately with a centimeter scale or comparator and use these relations to compute the spacing. This is hardly worth while, as exact comparator determinations of the reflection locations do not mean that one obtains correspondingly exact spacing determinations. Errors enter due to film shrinkage, specimen centering, absorption, etc., so that actually accuracies much better than $\pm 0.5\%$ are difficult to attain—unless one uses the high precision techniques of back reflection. Fortunately for our purposes we are not often interested in high accuracy; errors of 0.5% are usually not troublesome. Should some specialized studies, for example of solid solutions, require high precision, it can be attained by the use of special techniques.¹⁷

Much time in determining the spacings of powder lines can be saved by the use of special scales. Scales can thus be made up which read spacings directly, and with these adequate precision can be obtained. However, a new scale is then necessary for every camera diameter and for every wave length. In figure 4, d^* is shown to be $2 \sin \theta$. One can plot a scale of d^* and read the values from the film. By dividing into the wave length, the spacings are obtained. Figures 5 and 6 are charts for making such scales for cylindrical and flat films, respectively. A strip of paper is placed at the appropriate specimen-to-film distance and the intervals are marked off on the strip. This is done on both sides of the center. The scale is then centered on the film and values of d^* read off. A slide-rule division into λ then gives the spacings. Scales for odd specimen-to-film distances can be made in about five minutes each. Another advantage of this reciprocal-lattice method is that the scales are independent of wave length.

¹⁷ M. J. Buerger, *X-Ray Crystallography*. Wiley, New York, 1942, Chapter XX.

Determination of the relative intensities of the reflections is a more difficult task. To obtain even reasonably accurate intensities (assuming all experimental conditions have been fulfilled), a photometer must be used in combination with calibrating scales. One can, of course, abandon the use of film and use instead an ionization chamber or Geiger-Müller counter. Properly designed electrical circuits in combination with the ionization chamber or counter give readings which are proportional to the x-ray intensities. If such devices were combined with angular scales and self-recording apparatus, a more than acceptable substitute for films for powder work would be available.

A very simple way¹⁸ of obtaining intensities photographically is by the use of two films, one behind the other. The absorption by one film of the characteristic radiation being used is determined by experiments with strictly monochromatic radiation (crystal monochromatized—not merely filtered); thus, for Kodak no-screen film and copper $K\alpha$ radiation, one thickness of film absorbs about 75% of the incident beam. The second film will then correspond to an exposure of one-fourth of the first film, and by using the two films an intensity scale can be determined.

APPLICATIONS

Identification.—Every crystalline substance gives a unique diffraction diagram characterized by the spacings of the lines and their relative intensities. These quantitative data can, of course, be used as a means of identification. All that is needed is a catalog of patterns of known substances properly indexed. Such tables are available.¹⁹⁻²¹ In the card index system,²¹ the spacings for the three strongest lines for each substance are listed on separate cards and all cards are arranged in order of interplanar spacing. The procedure is usually to identify the component responsible for the strongest line, then eliminate all lines due to this component, then identify the strongest remaining line, etc. When the diagrams are complex and the lines overlap, elimination of lines must be done cautiously, making use not only of spacings but also of the relative intensities which are included on the cards.

The present card index system unfortunately is still in an experimental stage of development. (A recent valuable paper by Frevel²² points out

¹⁸ J. J. de Lange, J. M. Robertson, and I. Woodward, *Proc. Roy. Soc. London*, **A171**, 404 (1939).

¹⁹ J. D. Hanawalt, H. W. Rinn, and L. K. Frevel, *Ind. Eng. Chem., Anal. Ed.*, **10**, 457 (1938).

²⁰ L. K. Frevel, *Ind. Eng. Chem., Anal. Ed.*, **14**, 687 (1942).

²¹ *X-Ray Diffraction Data Cards*, Am. Soc. Testing Materials, Philadelphia, Pa.

²² L. K. Frevel, *Ind. Eng. Chem., Anal. Ed.*, **16**, 209 (1944).

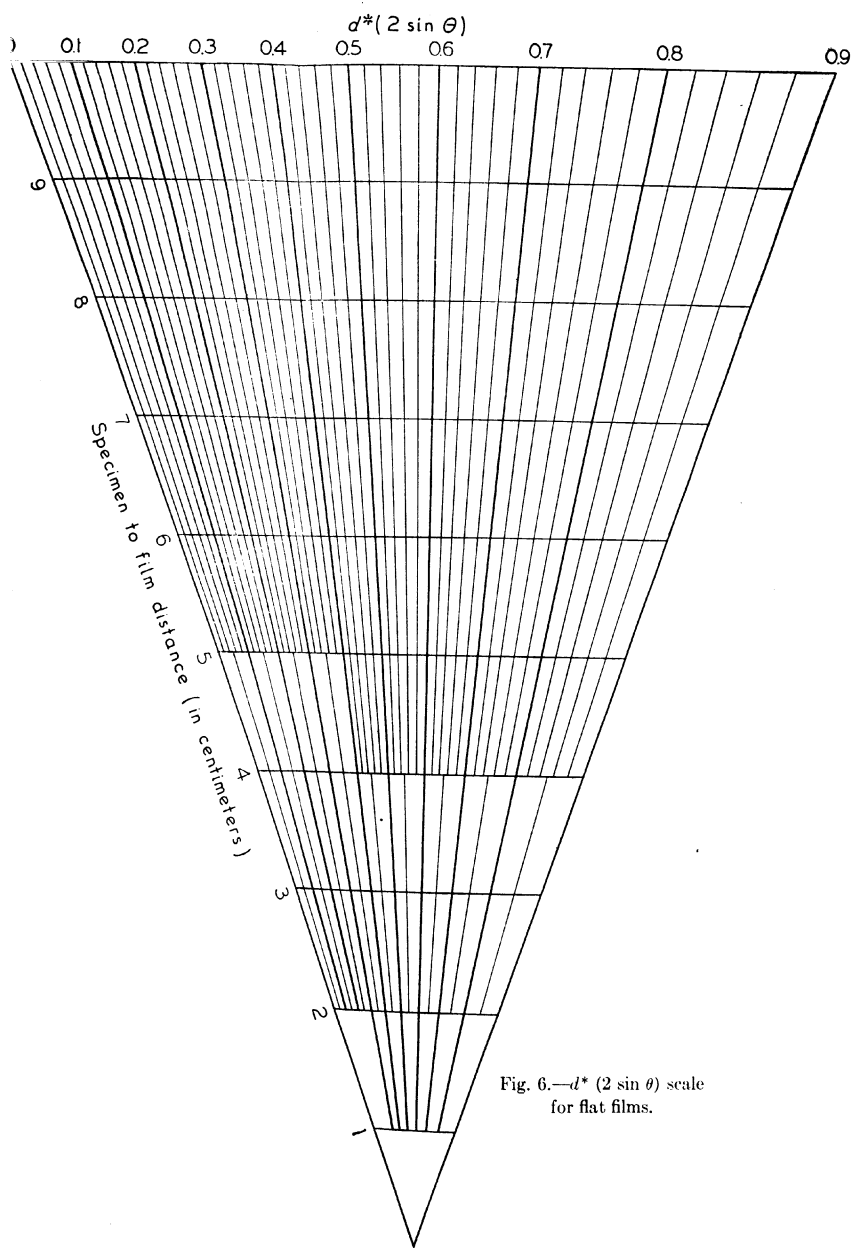


Fig. 6.— $d^*(2 \sin \theta)$ scale
for flat films.

some of the difficulties of the present system, and makes constructive suggestions for improving it.) While reasonable precautions were obviously taken in its preparation, nevertheless some of the data given are incorrect. One should be particularly careful in the use of the intensities, since one cannot always be certain what radiation was used in recording the diagrams or whether a slit or pinhole system was employed. However, the relative intensities of reflections not too far apart in spacing are reasonably independent of such considerations, and comparisons should thus be made only of neighboring reflections.

Precision Determination of Spacings.—There are some problems (the study of solid solutions, for example) in which slight changes in lattice constants must be followed. In such cases, one must study the back reflections occurring at Bragg angles close to 90° . It is easy to see why this is so from an inspection of Bragg's law. Near 90° , the sine curve flattens out and small changes in spacing, and therefore small changes in $\sin \theta$, correspond to large changes in θ ; in other words, the locations of the back reflections are very sensitive to changes in spacing.

It has also been shown¹⁷ that, by suitable extrapolations to $\theta = 90^\circ$, almost all errors can be eliminated. Absolute values of lattice constants to one part in 25,000 are possible, and relative changes as small as one in 50,000 can be detected.

Many organic crystals give poor lines in the back reflection range. Should high precision be desired in such cases, one can only use great care in determining the specimen-to-film distance, in eliminating centering errors, and in correcting for absorption and film shrinkage; but even with the greatest care it is extremely difficult to determine spacings with a smaller probable error than one part in 500 when the forward reflections only are used. It is almost impossible to determine by direct physical measurement the specimen-to-film distances. This should be determined by using a known material, sodium chloride, for example, as a calibrating material and computing the effective camera radius from the resulting diffraction pattern.

Particle Size Determination.—Just as for the optical case, the resolving power of the crystal as a diffraction grating is a function (among other things) of the size of the individual crystal grains. Particles larger than about 500 Å. give a resolving power not appreciably different from infinitely large crystals. With decreasing crystal size, the resolving power decreases and the diffraction lines become broader. This subject was first treated by Scherrer²³ and extensively developed by many other workers.

¹⁷ P. Scherrer, *Göttingen Nachrichten* (1918).

For recent reviews, see Patterson^{24, 25} and Clark.²⁶ It is possible from quantitative studies of the shapes of lines to determine particle size and shape. Such studies have been made, for example, of carbon blacks and of the size of the crystalline domains in fibers. In recent years, attention has been focused on studies of small angle scattering as a method of particle size study. Both discrete line diagrams²⁷ and continuous small angle scattering²⁸⁻³⁰ have given useful information about particle size and size of crystalline domains.

2. Single-Crystal Methods

It has already been shown that a single crystal arbitrarily oriented relative to an incident beam of monochromatic x-rays will generally give no reflected rays. If the crystal is rotated, then Bragg planes will move through the critical angles and reflections will flash out. In terms of the reciprocal lattice, points of the reciprocal lattice will pass through the sphere of reflection, and at the time of transit reflections will occur.

In all the common single-crystal techniques, the crystal is so adjusted that the axis of rotation (or oscillation) is parallel to a crystallographic axis, *i. e.*, parallel to a line of lattice points, because then all the reflection directions are elements of a discrete series of coaxial right circular cones the common axis of which is the axis of rotation. Moreover, there is then a very simple relation between the cone angles and the period (*not* an interplanar spacing—unless the axis happens to be an orthogonal axis) along the crystallographic direction.

A possible plane of lattice points is defined by any three noncollinear lattice points. Consider any crystallographic axis, that is, any line of lattice points. Pass a plane through it and rotate the plane with the line as axis. In one rotation the plane will pass through an infinite number of positions (if we consider the space lattice as infinite in all directions) in which it passes through other lattice points not on the axis. In each of these positions there is a set of crystallographic planes. The ensemble of all the sets of planes passing through the axis is called a zone and the axis is the zone-axis. Now consider the points of the reciprocal lattice which are reciprocal to the various planes of the zone. They will lie, by definition, on normals to the planes, and these normals will all be perpendicular to the

²⁴ A. L. Patterson, *Phys. Rev.*, **56**, 972 (1939).

²⁵ A. L. Patterson, *Phys. Rev.*, **56**, 578 (1939).

²⁶ G. L. Clark, *Applied X-Rays*. 3rd ed., McGraw-Hill, New York, 1940, p. 490.

²⁷ J. D. Bernal and I. Fankuchen, *J. Gen. Physiol.*, **25**, 111 (1941).

²⁸ A. Guinier, *Compt. rend.*, **204**, 1115 (1937).

²⁹ O. Kratky and A. Sekora, *Naturwissenschaften*, **31**, 46 (1943).

³⁰ I. Fankuchen and H. Mark, *J. Applied Phys.*, **15**, 364 (1944).

In figure 8, OA is the direction of the axis of rotation (and, of course, also the crystallographic axis, of periodicity a), C is the crystal and center of the sphere of reflection, and O is the origin of the reciprocal lattice. The layer of reciprocal lattice points corresponding to zone OA is perpendicular to OA and to the paper. Its trace is indicated by 0, and it contains reciprocal-lattice point of type $0kl$. Parallel to this equatorial plane at heights above (and below) it, $\zeta_1, \zeta_2, \zeta_3$, etc., where $\zeta_n = n\lambda/a$, are further planes of reciprocal-lattice points, the n th layer of which contains points of index type nkl . Their traces are indicated by 1, 2, 3, etc. As the crystal rotates or oscillates about an axis through C , the reciprocal lattice will rotate or oscillate about a parallel axis, OA . The layers, 0, 1, 2, etc., of reciprocal points will intersect the sphere of reflection in circles as indicated, and the

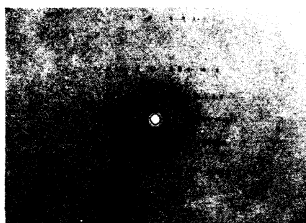


Fig. 9.—Oscillation diagram of menthol. Crystal monochromatized radiation.

points in these layers will pass through the sphere of reflection at locations of which the circles will be loci. The directions of the reflected beams will be from C to the points in the circles, *i. e.*, the reflected rays will be elements of a series of right circular cones. If a cylindrical film coaxial with the rotation axis is used to record the reflections, then the intersections of the cones with the cylinder will be a series of parallel circles. When the film is

straightened, the reflections will lie on a series of parallel straight layer lines (Fig. 9). If a flat film is used, the layer lines will be a series of hyperbolae. Consider the sequence of distances the various layers are from the equator (for the hyperbolae on a flat film, the measurements are made from the apices). Let EF be the intersection of the film (flat or cylindrical) with the plane of the paper. If s_n is the distance of the n th layer from the equator and r is the camera radius, then $\tan \omega_n = s_n/r$, where ω_n is the angle the elements of the n th cone make with the equator.

From figure 8, $\sin \omega_n = \zeta_n$ and $\zeta_n = n\lambda/a$. When these equations are combined:

$$a = \frac{n\lambda}{\sin \tan^{-1} s_n/r} \quad (6)$$

The value of s_n can be measured to an accuracy of about 1% if a cylindrical camera is used and the crystal axis properly adjusted to parallelism to the rotation axis. Thus the lengths of the three edges of any unit cell can be determined by rotating (or oscillating) the crystal about the three axes in

turn. It is important to notice that no interplanar *spacings* are necessarily determined by this method of measuring layer-line separations; what is determined is a periodicity along a crystallographic axis. This is true because the measurement is not of the location of one reflection but of the locus of many, namely, of a layer-line separation.

A. ROTATION AND OSCILLATION METHOD

When a crystal is rotated about an axis, the layer lines are well defined (if the crystal has been properly oriented) but densely populated with reflections. It is easy to determine the layer-line separation and consequently the periodicity, but difficult to identify (index) the individual reflections. That is because, in using a complete rotation, one reciprocal-lattice coordinate, the polar angle ϕ , has been lost. Consider, for example, the zero layer, *i. e.*, the $0kl$ reflections when the rotation is about the "*a*" axis. All $0kl$ points whose ξ value is less than two (the diameter of the sphere of reflection) will pass through the sphere during a complete rotation. Not only is this a large number, but for a given ξ value (*i. e.*, location on the film) there may be many different reflections. The disposition of reciprocal-lattice points in any layer will be at the corners of a parallelogram network. In indexing a single-crystal film, such a net is drawn for the layer in question to a convenient scale (10 cm. = unity is convenient) and the ξ values of the points of the net compared with the ξ values of the points on the film (see Bernal chart discussion below). If many points in the net are approximately at the same distance from the origin of the reciprocal lattice, then obviously unambiguous indexing becomes impossible.³¹

One solution of this difficulty is oscillation of the crystal about the axis over a small angular range, the magnitude of which will be an inverse function of the density of points in the reciprocal layers; 15° is a common value. Fewer points then pass through the sphere of reflection and the layer lines in the photographs are less densely populated. It then becomes possible to index the films unambiguously.

Bernal¹⁵ introduced the use of reciprocal charts for the interpretation of rotation and oscillation films (Figs. 10 and 11). The horizontal coordinate is ξ and the vertical ζ . To determine ξ and ζ for the spots on a film, the film is placed on the appropriate chart (the charts can be reduced or enlarged to take care of different camera sizes) and the coordinates read off directly. By means of these charts, layer-line separations and consequently axial periodicities can be determined very quickly; the period is given by $n\lambda/\zeta_n$ where ζ_n is the coordinate of the *n*th layer.

In indexing an oscillation film, it is simpler to consider the sphere of re-

³¹ M. J. Buerger, *X-Ray Crystallography*. Wiley, New York, 1942, Chapter VIII.

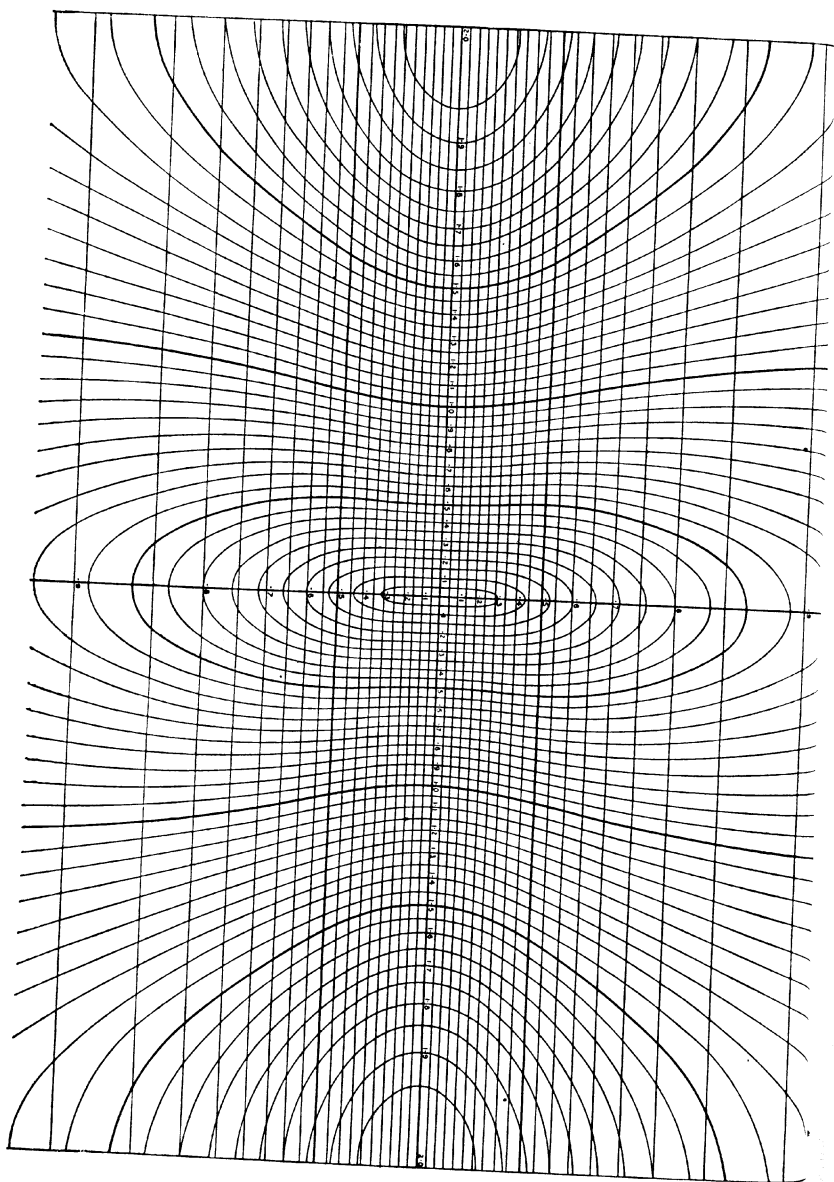


Fig. 10.—Bernal reciprocal chart for interpreting rotation and oscillation films from cylindrical cameras. Camera diameter 57.3 mm.

flection as oscillating (about OA as an axis, Fig. 8) and the reciprocal lattice as stationary. The film is indexed one layer at a time. For each layer

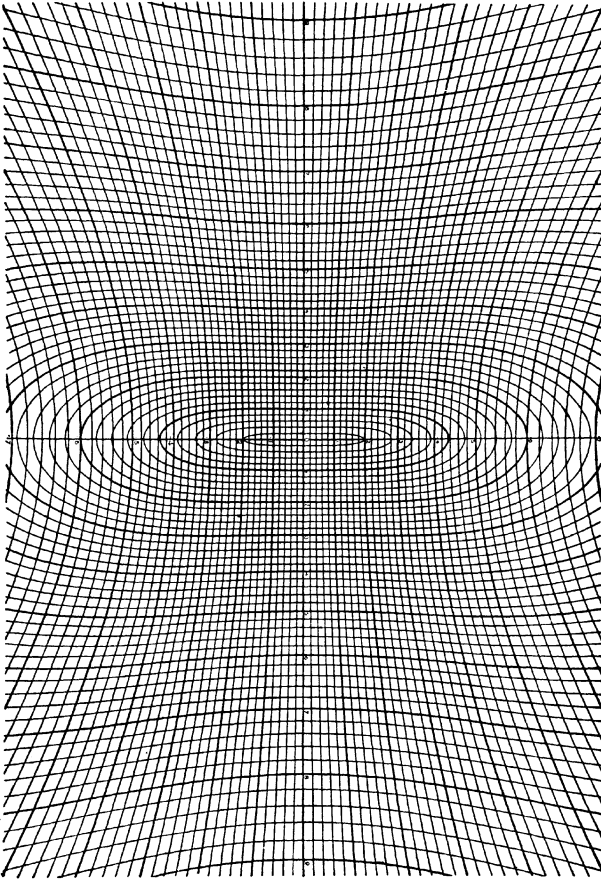


Fig. 11.—Bernal reciprocal chart for interpreting rotation and oscillation films from flat films. Specimen to film distance 5.00 cm.

the intersection of the layer with the extreme locations of the sphere of reflection will be a pair of lunes and those points of the reciprocal layer

which lie in the lunes will reflect during the oscillation. Lunes of appropriate size—depending on the ζ value of the layer line being indexed—are drawn on tracing paper and placed on a reciprocal net drawn to the same scale. For the zero layer, since it is always the same size ($\zeta = 0$), a permanent pair of lunes should be constructed on a nonshrinking transparent plastic base.³² By matching ξ value on film to ξ value on reciprocal net, the indices may be read off directly from each location in the reciprocal net which falls in the lunes and for whose ξ value there is a corresponding one on the film.

The same net can be used for all layers about a given axis. However, if the axis is not orthogonal, then the point about which the lunes rotate in the upper layers will be displaced from the origin of the plane reciprocal net by an amount which depends on the angle that a^* makes with " a " (for the case of oscillation about the " a " axis, for example) and on the ζ value of the layer.

B. WEISSENBERG TECHNIQUE

The Weissenberg and all other moving-film methods are designed to record one layer of the reciprocal lattice at a time. The principle is easy to visualize. Consider the oscillation method using the cylindrical camera. Let the crystal oscillate through 180° . The layer lines will be too densely populated to index if the usual stationary film is used. However, by inserting a cylindrical sleeve into the camera between the film and the rotation axis, it is possible to shield off most of the reflections so that only one chosen layer hits the cylindrical film. The sleeve has a slot cut almost all the way around; by making the insert slidable, the position of the slot can be adjusted so that only one chosen cone of reflections, *i. e.*, one layer, hits the film. The remainder of the reflections are blocked off by the opaque (to x-rays) shield. It is true that the layer line chosen will not be affected by this selective blocking off; it will be as densely populated as before. However, the individual reflections in the chosen layer do not occur at the same time; instead they flash out at different times during the revolution as the reciprocal-lattice points which correspond to them pass through the surface of the sphere of reflection. The apparatus is now modified by coupling the film cylinder to the oscillating mechanism so that the film slides up and down parallel to the axis of revolution as the crystal oscillates through 180° . As a result, the reflections, which for the stationary film were crowded into a line, are now spread out into a plane in such a way that no two reflections can fall in the same place. This permits unequivocal

³² M. J. Buerger, *op. cit.*, Fig. 105.

indexing; actually, with a little experience, one can index a Weissenberg film merely by looking at it and counting!

Crowfoot³³ and Buerger,³⁴ using the reciprocal lattice, have treated the indexing of Weissenberg films. They have derived charts which are really the equivalent, for the Weissenberg method, of the Bernal charts for the fixed-film techniques. The film is again placed upon the appropriate chart (here one must use a chart of the correct camera size and coupling constant), and reciprocal lattice coordinates in the plane net are read off directly.

The moving-film techniques are the most elegant techniques in x-ray diffraction for obtaining data for structure analysis. By their use, the essential x-ray data can be collected in a minimum of time, and certainly the indexing of the films requires little labor and is less likely to permit of erroneous identification of the reflections.

A fiber diagram is similar in character to a rotation diagram; and what has been said of the rotation method applies to the interpretation of fiber diagrams. The fiber period can be determined unequivocally; but most attempts to carry the interpretation further have only led to controversy.

As in so many other scientific techniques, the difference between success and failure in the study of single organic crystals with x-rays often lies in apparently small and trivial things. Thus, the organic chemist, accustomed as he is to crystallization processes, may be unable to produce crystals suitable for x-ray and optical studies. The reason is obvious—the chemist uses crystallization procedures not to produce good crystals but rather to produce pure materials, and the conditions for producing pure crystals are not necessarily those which result in good crystals.

Usually, slow crystallization is desirable from the viewpoint both of the x-ray worker and of the optical microscopist, since this results in larger and more perfect crystals. It is not necessary to use large amounts of solute. Suitable crystals of most organic materials can be obtained by slow cooling of a warm saturated solution. A convenient way to obtain such crystals is to place about one milliliter of a warm saturated solution in a small test tube which is inserted through a hole in a large flat cork and then floated in a beaker of hot water. As the water cools, crystals appear and grow. When the crystals are of suitable size, the process can be stopped by removing the surplus solution.

Special techniques for recrystallizing small quantities of materials and for handling very tiny crystals have been developed in Bernal's laboratories in Cambridge and London, particularly in connection with the studies on

³³ D. Crowfoot, *Z. Krist.*, **A90**, 215 (1935).

³⁴ M. J. Buerger, *op. cit.*, Chapters XI–XV.

sterols,³⁵ proteins,³⁶ and viruses.²⁷ Perutz³⁷ has described one convenient manner of recrystallizing small amounts of material in such a way as to obtain clean, dry crystals. Another simple technique consists of spacing two microscope slides about one millimeter apart by means of four small lumps of Plasticine placed at the corners. A drop of warm saturated solution is inserted into the space between the slides. The subsequent crystallization as the solution cools and evaporates can be conveniently followed under the microscope. It can, furthermore, be stopped at any stage by inserting a fine capillary into the drop and sucking out the surplus solution.

A good way to mount crystals for both x-ray and goniometric studies is to attach them to fine Pyrex glass fibers of appropriate diameter. If the crystal is of the right size for x-ray work, it is too small to mount with the naked eye and should be mounted under the microscope. The fiber is embedded in a small lump of Plasticine which in turn is fastened to a rod which serves as a handle. The fiber should protrude about one-fourth of an inch from the Plasticine. The end of the fiber is wet with dilute shellac (this dries slowly enough to permit of reasonable ease in mounting) and brought at the desired angle in contact with the crystal. If the correct size of fiber has been chosen, then the shellac will be dry enough in a minute or so to permit the crystal to be mounted by means of the Plasticine on the goniometer head of the x-ray camera or optical goniometer. In adjusting the crystal on the goniometer head, it should first be set approximately at the center of the adjusting arcs and then centered roughly on the axis of rotation. The arcs are then adjusted until the desired crystal direction is parallel to the axis of rotation. When this has been attained, the crystal should be recentered without disturbing the angular adjustment.

Of course this assumes recognizable, good crystal faces. When good enough faces are not found, then only an approximate orientation can be achieved. The resulting x-ray diagrams will be distorted, *i. e.*, the layer lines will not be straight. The necessary corrections can then be made³⁸ from a study of the observed distortions.

C. APPLICATIONS

Determination of Molecular Weights.—Because all the early x-ray work was done on materials of known constitution, the possibility of using x-ray diffraction measurements to determine molecular weights has been

³⁵ J. D. Bernal, D. Crowfoot, and I. Fankuchen, *Trans. Roy. Soc. London*, **A239**, 135 (1940). D. Crowfoot in *Vitamins and Hormones*, Vol. II, Academic Press, New York, 1944, p. 409.

³⁶ I. Fankuchen, *Ann. N. Y. Acad. Sci.*, **41**, 157 (1941).

³⁷ M. Perutz, *Z. Krist.*, **A96**, 328 (1937).

³⁸ O. P. Hendershot, *Rev. Sci. Instruments*, **8**, 436 (1937).

largely overlooked. In many branches of organic chemistry, for example in the study of biological materials, the molecular weight of the material may be either unknown or known with low accuracy. It is then possible by means of x-rays (providing, of course, that the substance is crystalline) to determine the molecular weight (or, at the worst, a small integral multiple of it). Moreover, only a very small amount of material is necessary; a single crystal weighing 10^{-6} gram can be used for such a determination.

The unit cell of a crystal must contain a whole number of molecules. The number is usually small and is further limited by the symmetry of the crystal. Thus the number of molecules in an orthorhombic crystal will *almost* always be a power of two, in the tetragonal a multiple of four, etc.

From single crystal x-ray diagrams, one can determine unequivocally the size and shape of the unit cell. The volume can then be computed; by one of the well-known methods, the density is determined; and the product of volume and density gives the mass of the unit cell. The mass of the hydrogen atom of atomic weight 1.008 is 1.66×10^{-24} gram. Therefore, the weight of the cell contents referred to oxygen as 16 can be obtained by dividing by 1.65×10^{-24} . Since $1 \text{ \AA.} = 10^{-8} \text{ cm.}$, $1 \text{ \AA.}^3 = 10^{-24} \text{ ml.}$ The 10^{-24} is eliminated if the computation is carried out in angstrom units, and the equation for the molecular weight, $M.W.$, of the material becomes:

$$\frac{Vd}{n \times 1.66} = M.W. \quad (7)$$

where n is the number of molecules in the unit cell. For V in (kX units)³ use 1.65 instead of 1.66.

Often n turns out to be the number of general positions in the space group. Its value can be obtained directly if one has a very approximate idea of the molecular weight. The approximate value is put into equation (7) and the equation is solved for n . The value for n will be close to one of the possible integral values if the approximate molecular weight used was not too far off. The integral value for n is then used to compute the x-ray molecular weight. This assumes that no solvent of crystallization is present. If the crystal contains solvent of crystallization, then if $M.W.$ is known, equation (7) can be used to compute the quantity of solvent present.

Determination of Other Molecular Characteristics. Complete Structure Determination.—If a complete structure determination can be made, then the entire stereochemistry of the molecule becomes known. It is not, however, always necessary to attempt a structure determination to obtain useful information. Non-x-ray data can be applied to the problem. Optical measurements with the polarizing microscope are very helpful. The optical characteristics of crystals reflect the anisotropy of the molecules which make up the crystal. Thus, flat molecules are usually optically nega-

tive and elongated molecules positive.³⁹ From rough ideas of the molecular size and the optics, one can guess pretty well the molecular arrangement within the unit cell and often decide between alternative stereochemical configurations. Often the absolute values of the cell constants place limitations on molecular dimensions. Sometimes the symmetry of a molecule can be determined without a complete structure analysis.

To make a complete structure determination, the intensities of the x-ray reflections must be used, for these are functions of the atomic distribution within the unit cell. It should be noted that hydrogen atoms are not located directly although their locations can usually be inferred from those of the other atoms.

The intensity of a reflection (hkl) is proportional to the square of the amplitude, F_{hkl} , of the reflected wave. The scattering of x-rays by an atom is really a scattering by the electrons within the atom. When the direction of scattering is forward, all the electrons in an atom scatter in phase and an atom of atomic number Z will scatter as though its Z electrons were all concentrated at a point. When the scattering is in any other direction, the electrons (which in all atoms are distributed over a radius roughly of the same order of magnitude as the wave length of the x-rays) do not scatter in phase and the amplitude of the resultant wave is less than that of Z electrons at a point.

It can be shown that the effective scattering of a given atom is a function of $\sin \theta/\lambda$ and this function has been evaluated both theoretically and experimentally for most atoms.⁴ The shapes for all atoms are similar. At $\sin \theta/\lambda = 0$, the curves (f curves) start at Z (or $Z \mp n$ if the scattering unit is an ion of charge of $\mp ne$), then fall steadily, and finally flatten out for large $\sin \theta/\lambda$. It is to be noted that the curves for atoms of low atomic number fall off more rapidly than those for atoms of high atomic numbers. This means that, at large Bragg angles, most of the scattering can be attributed to whatever heavy atoms are present. The f values of the constituent atoms and their distribution in the unit cell determine the amplitudes, F_{hkl} , of the different reflections. A proposed structure can be tested by computing the scattering in various Bragg directions and then comparing the computed with the observed intensities.

For each set of reflecting planes, (hkl), there is a structure factor, F_{hkl} , which is a measure of the efficiency of that set of planes in reflecting x-rays. This factor arises because the scattering by the different atoms (or, more fundamentally, by the electrons) is not necessarily in phase and each reflection is therefore weaker than it would have been if all the atoms had been located in the reflecting planes and thus scattered in phase. F_{hkl} is

³⁹ R. C. Evans, *Crystal Chemistry*. Cambridge Univ. Press, London, 1939, p. 273.

the amplitude of the x-ray wave reflected by a given set of planes in terms of the scattering of the same lattice with just one electron at each lattice point as unity.

Consider a unit cell of dimensions a, b, c with n atoms per unit cell. Let the coordinates of the p th atom be $x_p y_p z_p$ when referred to the sides of the unit cell as the coordinate system (not necessarily an orthogonal system). Thus, x can vary in magnitude from 0 to a , y from 0 to b , and z from 0 to c . Then it can be shown that:

$$F_{hkl} = \sum_p f_p e^{2\pi i(hx_p/a + ky_p/b + lz_p/c)} \quad (8)$$

Each term in the above summation is merely the contribution of the corresponding atom to the amplitude of the scattered wave. For practically all organic crystals, the intensity of a reflection will be proportional to the square of F . A geometrical factor which is a function of θ also enters into the expression for the intensity. This factor depends on the experimental setup for its form (see Bragg⁴⁰).

The above expression makes possible computation of the amplitudes and therefore the relative intensities of the reflections to be expected from any assumed structure. Actually, the most common way of working out structures is to assume structures, note which gives the best agreement between observed and computed intensities, modify the assumed structure slightly, recheck the intensities, etc.

There is an elegant way of utilizing the x-ray data in working out structures, namely, the method of Fourier series. The density, ρ , of electrons in a crystal is a regular periodic function in space. It can, therefore, be represented by a three-dimensional Fourier series. It can be shown that the coefficients of this series are the F_{hkl} values. The expression is:

$$\rho(xyz) = \text{constant} \cdot \sum_{-\infty}^{+\infty} \sum F_{hkl} e^{-2\pi i(hx/a + ky/b + lz/c)} \quad (9)$$

When the crystal has a center of symmetry and the origin of the coordinate system is placed at a center of symmetry, a great simplification results. The F 's become real numbers, $F_{hkl} = \mp F_{\bar{h}\bar{k}\bar{l}}$ and:

$$\rho(xyz) = \text{constant} \cdot \sum_{-\infty}^{+\infty} \sum F_{hkl} \cdot \cos 2\pi(hx/a + ky/b + lz/c) \quad (10)$$

Even this form is too involved for frequent use. If the F 's for one principal zone are used, the $hk0$ reflections for example, then the series simplifies to a two-dimensional series. This series represents the density of the projection of the cell contents onto the xy plane.

⁴⁰ W. L. Bragg, *The Crystalline State*. Macmillan, New York, 1934, Chapter IX and Appendix V.

$$\rho(xy) = \text{constant} \cdot \sum_{-\infty}^{+\infty} \sum_{-\infty}^{+\infty} F_{hk0} \cos 2\pi(hx/a + ky/b) \quad (11)$$

Similar expressions can be written for $\rho(xz)$ and $\rho(yz)$. By projecting onto a chosen line instead of onto a plane, corresponding one-dimensional series can be obtained, that is, expressions for $\rho(x)$, $\rho(y)$, and $\rho(z)$.

Now $\rho(xy)$ is the projection of the electron density, *along* the *c*-axis onto the *c*-face. When a two-dimensional summation (or a plane section of a three-dimensional summation) is evaluated, it is usually plotted by using contour lines. A Fourier plot will resemble the conventional topographic contour map, the peaks representing regions of high electron density, hence atoms. The complete data for such a summation are contained on one Weissenberg film, the equatorial layer of a "*c*"-axis Weissenberg film for the case in question.

Methods have been worked out^{41, 42} for rapidly computing such two-dimensional series. With such methods, an average two-dimensional series can be evaluated in 1 day, a one-dimensional projection in about 5 min.

There is, of course, a catch in the above discussion; otherwise, any crystal for which the x-ray diagrams could be obtained could have its structure determined directly. The trouble arises because experimentally the x-ray intensity is observed, that is, a quantity proportional to $|F|^2$. From this, we can determine $|F|$, the absolute magnitude of F . However, F is in the general case a complex number (Eq. 8), and at best a real number whose sign is either plus or minus. Lacking the phase angle of the complex number (or the sign of the real number) we *do not* know the coefficients of the Fourier series; we only know their magnitudes. Generally, an approximate knowledge of the structure (or a good guess) is first required. This can be used to compute phase angles or determine signs which then can be used to compute the Fourier series. The method is really one of successive approximations.

Failing such advance knowledge of the phase angles (which may be obtained from comparison with similar substances of known structure, application of the principles of crystal chemistry, or perhaps an inspired guess), the Fourier expressions for *electron density* cannot be used and recourse must be made to other methods of analysis.

Patterson^{43, 44} investigated the question of how much information could be obtained from a knowledge only of F^2 values. He was able to give a most useful physical interpretation to the summation of a three-dimen-

⁴¹ H. Lipson and C. A. Beevers, *Proc. Phys. Soc. London*, **48**, 772 (1936).

⁴² J. M. Robertson, *Phil. Mag.*, **21**, 176 (1936).

⁴³ A. L. Patterson, *Z. Krist.*, **A90**, 517 (1935).

⁴⁴ A. L. Patterson, *Z. Krist.*, **A90**, 543 (1935).

sional Fourier series identical with equation (9) save for the replacement of F_{hkl} by $|F_{hkl}|^2$. The sum of such a series is denoted by $P(xyz)$. (There are also the corresponding two- and one-dimensional series.) Consider an atomic distribution of N atoms in a unit cell and let it be represented by $\rho(xyz)$ of equation (9). By starting from each atom in the unit cell, vectors may be drawn to every atom in the cell (including the identity vector, *i. e.*, to itself). There will be N^2 such vectors. The arrow end of each vector is weighted by a weight proportional to the product of the atomic numbers (*i. e.*, the product of the x-ray scattering efficiencies) of the atoms it connects. An origin is selected and all the weighted vectors transferred to it. This distribution of products of atomic numbers is then represented by $P(xyz)$ just as $\rho(xyz)$ represents the original distribution of atoms.

It is apparent that the interpretation of this Patterson summation will generally be a difficult task: Whereas the F summation contains N comparatively well-defined peaks, the F^2 summation will contain in the same volume N^2 peaks which must superimpose to a great degree. Harker⁴⁵ has provided a modification of the F^2 summations which makes the interpretation somewhat easier in many cases.

If the crystal contains a few heavy atoms, the sorting-out process is much simplified. Since each vector in the Patterson diagram is weighted proportionately to the product of the atomic numbers of the atoms it connects, the vectors connecting the few heavy atoms with each other produce heavy peaks. Their identification usually permits the determination of the coordinates (parameters) of the heavy atoms. This often constitutes enough knowledge of the approximate structure to enable the computation of phase angles of the F 's and the evaluation of the F series. A review paper by Robertson⁴⁶ provides a most comprehensive and clear discussion of the application of Fourier series to the determination of molecular structures. Recently, Harker and Kasper⁴⁷ showed that the possibility exists of determining some of the phase angles directly; and Gillis⁴⁸ has shown that the method gives useful results.

VII. EXAMPLES OF USES IN ORGANIC CHEMISTRY

Some examples of the use of x-ray diffraction as a tool in organic chemistry are given. Bernal *et al.*⁴⁹ made an x-ray and optic study of steroid structures. It was early in this work that Bernal⁴⁹ showed that the gen-

⁴⁵ D. Harker, *J. Chem. Phys.*, **4**, 381 (1936).

⁴⁶ J. M. Robertson, in *Reports on Progress in Physics*. Vol. IV, The Physical Society, London, 1938, p. 332.

⁴⁷ D. Harker and J. S. Kasper, *Acta Crystallographica*, **1**, 70 (1948).

⁴⁸ J. Gillis, *Acta Crystallographica*, **1**, 76, 174 (1948).

⁴⁹ J. D. Bernal, *Nature*, **129**, 277 (1932); *J. Soc. Chem. Ind.*, **51**, 466 (1932).

erally accepted stereochemical configuration of Windaus could not be right and that the (now accepted) structure of Rosenheim and King and of Wieland and Dane was probably correct. This result was obtained merely from a cell determination using single-crystal techniques combined with polarizing microscope studies. The Windaus molecule could not fit into the observed unit cells—hence, it could not be right. As the work on sterols continued, it was evident that it would be premature to attempt a structure determination on any single sterol. A systematic study was then undertaken of every steroid which could be obtained—about 100 in all. As a result, it was found possible to classify the steroids on the basis of the x-ray and optic studies in such a way that rapid identification of any one of them could be made. Several of the “sterols” were thus found to be mixtures of individual sterols. As a result of x-ray molecular weight computations, a few of the sterols were found to have incorrect stoichiometric formulas. These are but a few of the chemical findings of this work.

Robertson⁴⁶ has carried out the best and most extensive studies of molecular compounds using Fourier methods. The finest example to date of his work is that on the phthalocyanines.⁵⁰ Here, *by x-ray alone*, he was able to work out completely the structure of a substance about as complex as chlorophyll. This was done actually in two different ways. Phthalocyanine crystallizes in isomorphous structure either with hydrogen atoms (which do not scatter appreciably) or with a nickel atom at its center. The geometry of the x-ray diagrams for both materials is identical, but the relative intensities of the reflections are very different. Now the only difference in the scattering of the two unit cells is the scattering of the nickel atom. Its location (and therefore contribution to the amplitude of the scattered waves) was known to be, on x-ray grounds, at a center of symmetry. This made it possible to determine the signs of the structure factors (they were real because of the presence of a center of symmetry); and the resulting Fourier summation revealed the entire stereochemistry of the molecule.

In the case of platinum phthalocyanine, the structure could be worked out in detail for another reason. The platinum atoms are located at centers of symmetry and their contribution to the structure factors, F_{hkl} , could be computed. Because of the massiveness of the platinum, its contribution was sufficient to determine the sign of all but a few of the weakest reflections.

The phthalocyanines illustrate two general methods of working out structures—the use of isomorphous substitutions and of heavy atoms. These can often be used in studying organic structures.

⁵⁰ J. M. Robertson, *J. Chem. Soc.*, 1935, 615; 1936, 1195. J. M. Robertson and I. Woodward, *ibid.*, 1937, 219; 1940, 36.

Hargreaves and Taylor,⁵¹ in an interesting paper, give several illustrations of the utility of the x-ray approach. In one of the examples, calycanine, $C_{16}H_{10}N_2$, is studied. By means of x-rays it was possible to decide which of 13 alternative stereochemical configurations was the most likely.

In a brief study of biotin,⁵² the use of x-rays confirmed the molecular-weight determination and suggested a flat molecule.

X-ray work has been useful in the study of proteins. Here, molecular weights must be determined (except for rare cases like hemoglobin) not by chemical analysis but by rather involved and complicated processes, the ultracentrifuge, diffusion measurements, etc. X-ray methods have resulted in reliable molecular weights³⁶ when good crystals and hence good diagrams (Fig. 2D) could be obtained.

Virus proteins have also been studied.²⁷ It was possible to obtain exact measurements of the diameter of the virus molecules. Low-angle scattering studies threw much light on the character of interparticle forces in colloidal preparations. It was also demonstrated that the virus molecules themselves had a regular internal structure, that is, they were tiny crystals themselves.

Many more examples could be cited of the utility of x-rays as a tool in chemistry. The illustrations given above have not included its use for identification by the powder method because that is a common and generally accepted technique. But enough have been given, it is hoped, to demonstrate that the x-ray diffraction method is a powerful tool in chemistry and one which should be more widely used.

⁵¹ A. Hargreaves and W. H. Taylor, *J. Sci. Instruments*, **18**, 138 (1941).

⁵² I. Fankuchen, *J. Am. Chem. Soc.*, **64**, 1742 (1942).

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ELECTRON DIFFRACTION

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I. INTRODUCTION

Electron diffraction as a technique in organic chemistry is concerned chiefly with the determination of the geometric structures of molecules. The information directly afforded is the locations of the individual atoms from which may be calculated the distances between chemically bonded atoms, the angles between bonds, the characteristic configurations of typical valence bond structures, the closest approach between nonbonded atoms, and any other structural parameters of interest such as those distinguishing geometric isomers. Such studies with electron diffraction are ordinarily applied only to the vapor state because of the low penetrating power of electron beams in solids. Molecules in the crystalline state are usually studied by x-ray diffraction methods although both methods may be applied to fine crystalline powders. Electron diffraction by massive solids is used when the surface properties are to be investigated and often leads to a chemical identification of adsorbed materials or surface layers differing

from the substrate¹; this field will not be discussed here. The technique described in the following pages is intended to yield the maximum information about molecular parameters. Simplifications are introduced at certain stages in the treatment which would not be allowed in a thorough test of diffraction theory but which do not seriously affect the locating of the atomic scattering centers and are required for convenient application of the method to structural problems.

The experimental procedure involves the operation of high-vacuum, high-potential and vacuum-tube control equipment but only in more or less standard forms already applied to other experiments. Thus, the power supply for an x-ray tube can be used for the high-potential source. Although no commercial electron diffraction camera for gases has been offered for sale in this country, nearly all of the camera accessories can be assembled from commercial items, while the camera can be constructed in any good machine shop. The essential features of the design are given below. The operator will benefit considerably from some acquaintance with established principles in high-vacuum and high-potential technique.

The interpretation of the diffraction patterns and the limitations on the applicability of the method are discussed in the last two sections (pages 1119 and 1136). It should be noted at the outset that, as a completely independent method of structure determination, electron diffraction is very severely limited. Usually only three molecular parameters can be determined independently, and quite simple molecules would present insuperable difficulties in the absence of supplementary data. For example, the CCl_3 group in chloroform is fixed by six parameters, three carbon-chlorine and three chlorine-chlorine distances; however, with the assumption of an axis of trigonal symmetry supported by spectroscopic data on groups of this type, only two distinct distances occur. Again, in benzene, the six carbon atoms are fixed by twelve parameters, but on the basis of the chemical evidence for the equivalence of the six atoms its structure may be confidently discussed in terms of a six-membered ring fixed by only two parameters. Supplementary data bearing on the symmetry of the molecule are especially useful in the application of the electron diffraction method, but the nature of the supplementary assumptions should be clearly indicated in stating diffraction results. Another kind of assumption also extends the value of electron diffraction results. The smallness of the variations observed in bond lengths and angles for given bond types among simple organic molecules makes it probable that the characteristic values for these bonds also occur in larger molecules too complicated for direct diffraction studies.

¹ G. Finch, *Trans. Faraday Soc.*, **31**, 1051 (1935); *Ergeb. exakt. Naturwiss.*, **16**, 352 (1937).

II. PREPARATION OF DIFFRACTION PHOTOGRAPHS

The diffraction effect is observed by causing a beam of electrons to traverse the vapor under investigation and recording the scattered electrons on a photographic plate. The production of usable patterns requires all of the electrons to have nearly the same velocity, the unscattered beam to strike the recording plate in a very small area, and the scattering gas to meet the beam through a very short section of its path. Because of the relatively high electron-scattering power of air, the whole electron path is enclosed in a vacuum chamber. The diffraction camera consists of an electron tube for producing the beam, a magnetic lens or other means of collimating the beam, a specimen section where the gas stream crosses the electron beam, a photographic plate holder, and the auxiliary equipment including the vacuum pumps and gages and the electrical power supply. Many of the constructional details may be varied according to convenience and available shop facilities. The camera used by the author and illustrated in figure 3 (page 1113) embodies a sectional style arranged for easy access to the various parts; but other cameras of different design have been used successfully. In the following paragraphs are discussed the features essential to obtaining good diffraction photographs.

1. Electron Beam

The path of the electron beam is shown in figure 1. The electrons liberated from the heated filament on the left are accelerated to the anode by a

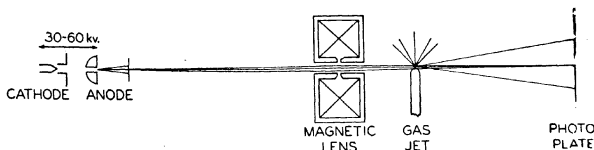


Fig. 1.—Path of electron beam.

direct-current potential of 30 to 60 kilovolts. The beam, limited in size by a small hole in the anode, passes on through a magnetic lens and through the jet of vapor to the photographic plate. The requirement mentioned above of uniform velocity for the electrons is met by providing an accelerating voltage which has a maximum variation of about 0.5%. The power supply for the accelerating potential may be assembled quite simply from a high-voltage transformer, one rectifier tube, and one filter condenser. Since the high-voltage current required is only about one milliamper, including the drain through a high resistance for measuring the potential, a

filter condenser of one-half microfarad has enough capacity to pass only a small ripple voltage (less than 0.1% at 40,000 volts) even with the half-wave rectifier indicated in figure 2. A more serious difficulty often arises from voltage fluctuations in the alternating-current line supplying the high-potential transformer. If these fluctuations are as large as 1%, a low-voltage stabilizer must be used in the supply line or a high-voltage stabilizer may be inserted at the position shown in figure 2. Several stabilizer circuits suitable for 50 kilovolts were devised.^{1a} The best type (not yet published) uses a control tube in series in the high-potential output with the plate potential varied to absorb voltage fluctuations. The actual value of the accelerating potential determines the electron wave length and should be observed at the time the diffraction exposure is

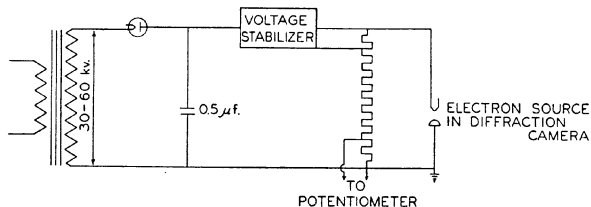


Fig. 2.—Simplified diagram of power supply.

made. A high resistance (fifty megohms or more) is connected across the high potential next to the electron diffraction tube, and the potential drop across a small series resistance at the grounded end is read on a potentiometer. Since the high and low resistances are of high quality, wire-wound, mounted in oil, and calibrated, the observed potential multiplied by the resistance ratio measures the full accelerating potential. The effective wave length (in centimeters) is then calculated by the relation:

$$\lambda = (150/V)^{1/2} \cdot h/(em_0)^{1/2} (1 + eV/600m_0c^2)^{-1/2}$$

in which V is the accelerating potential in volts, and h , e , m_0 , and c are Planck's constant, the electronic charge, mass of the electron, and the velocity of light, respectively. When the current values of the physical constants² are used, the relation (expressed in angstrom units) becomes:

$$\lambda = (150/V)^{1/2} \cdot 1.002 \cdot (1 + 9.777 \cdot 10^{-7}V)^{-1/2}$$

If the available potential divider and potentiometers are not good enough for determining absolute potential differences, it would be almost as satisfactory to use the potentiometric measurement only to check on the con-

^{1a} A. W. Vance, *RCA Review*, **5**, 293 (1941).

² R. T. Birge, *Phys. Rev.*, **52**, 241 (1937); **60**, 785 (1941); **61**, 204 (1942).

stancy of the potential and to calibrate the electron wave length by taking diffraction photographs of a known crystalline substance, such as gold foil. In the case of gold or any other metal whose crystal structure is based on the cubic lattice, the wave length is calculated from the Bragg relation, $\lambda = (2a_0 \sin \theta/2)/(h^2 + k^2 + l^2)^{1/2}$, in which a_0 is the length of the edge of the unit cell (4.070 Å. for gold³), θ is the observed angle between the incident ray and the scattered ray producing one of the diffraction rings, and h , k , and l are the integers which distinguish the reflecting crystallographic planes and which, for face-centered structures, as in gold, may have any combination of values for which the three integers are all even or all odd.

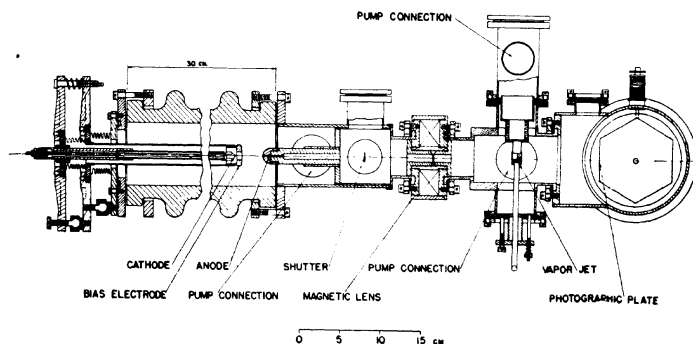


Fig. 3.—Sectional view of gas diffraction camera.

The second requirement for good diffraction patterns is that the diameter of the undeflected electron beam be small when it reaches the photographic plate since the pattern becomes more diffuse when the size of the beam is increased. The use of a magnetic lens is illustrated in figure 1, and the location of the lens in relation to the other parts of the diffraction camera is shown in figure 3. The electrons coming through the small hole in the anode (about 0.5 mm. in diameter) travel on divergent paths until they enter the field of the lens where they are caused to converge. The lens consists of about 2000 turns of wire enclosed in an iron sheath having a gap on the inside. Although the shape and size of the gap affect the focal length, it is very readily adjusted by varying the current through the winding until the electron spot observed on a fluorescent screen at the position of the photographic plate has its minimum size. When the beam is focused in this way, the minimum size of the electron spot is determined by the diame-

³ M. C. Neuberger, *Z. Krist.*, **93**, 1 (1936).

ter of the hole in the anode and the ratio of the lens-plate distance to the lens-anode distance. Both these factors should have the smallest practical size. The diameter of the anode hole should not be less than a few tenths of a millimeter in order that the electron current in the beam be not too small. The ratio of the image and object distances of the lens is approximately one in the camera illustrated, and the electron spot on the plate and the anode hole have the same size. Auxiliary diaphragms are necessary to shield the plate from stray electrons, which arise from scattering at the edges of the anode hole and improper focusing of electrons passing near the edge of the lens. Figure 1 shows a diaphragm following the anode; this diaphragm is effective in eliminating most of the background scattering and has a hole of 1.5 diameter; a second diaphragm is attached to the gas nozzle.

It is possible to dispense with the lens and to collimate the beam using the holes in two diaphragms. The maximum size of the electron spot on the plate is then determined by the cone subtended by the holes in the diaphragms; and the angle of the cone must be small enough to give the electron spot the desired small size. The disadvantage arises in the reduction of the electron-beam current which follows reduction in the cone angle.

The electron-beam current is enhanced if the space current between the cathode and anode is concentrated on the opening in the anode. An auxiliary cylindrical electrode to assist in focusing the space current is shown in figure 1. The electron source is a V of 0.007-inch tungsten wire with the tip in the opening of a cylinder (10 mm. diameter and length) which is made negative with respect to the filament by 100 to 200 volts. With 40,000 volts applied between the filament and anode and a space current of 30 microamperes, the beam current may be as large as ten microamperes. The exposure of the gas stream to the electron beam is controlled from the outside by a rod acting through a flexible metal bellows to retract a movable diaphragm. This mechanical shutter is simple and convenient; and because precise timing of the exposure is usually not important it has no disadvantage in comparison with the use of magnetic or electric fields for deflecting the beam.

2. Specimen

Gases as diffraction specimens are brought into the electron beam through a small hole in the end of a nozzle just under the beam. Since the beam should meet the specimen only in a very small region, special precautions are necessary to keep the gas from spreading through the camera and meeting the beam all along its path. An earlier method provided for condensing much of the gas on a metal surface mounted close to the electron beam on the side opposite the gas jet and cooled with liquid air from the outside

of the camera. In the present arrangement, shown in figure 3, the gas jet is connected with a tube leading into a high-vacuum pump so that the gas path through the camera is entirely enclosed except for the two openings required for letting the electron beam pass through the gas stream. The sample of gas would be confined most effectively if windows could be used which would scatter electrons less effectively than the gas does; but because of the high density of the window material relative to the gas such windows would have to be extremely thin if the amount of material in the path of the beam through the windows were less than that in the beam path through the gas.

Since the exposure time for the gas diffraction photograph is never more than a few seconds, the gas is introduced in a spurt timed to the opening of the shutter controlling the beam. The pressure of gas where the diffraction occurs cannot be measured; but, if the nozzle is connected to a reservoir of gas on the outside from which the flow is controlled by the momentary opening of a stopcock, the pressure in the reservoir required for good diffraction photographs ranges from 50 to 200 millimeters. If the substance under study has a vapor pressure at room temperature which is higher than this range, the temperature of the reservoir is adjusted in a cooling bath. This method is convenient for materials of boiling points down to about -100°C. ; in such cases, the reservoir may be a 50-milliliter glass bulb connected through a stopcock and a standard taper to the metal tube on the camera. Into the bulb is condensed about 0.5 milliliter of liquid, an amount more than sufficient for 25 or 30 photographs. For gases of extremely low boiling point, the sample may be handled as a gas in a 500-milliliter bulb with an initial pressure of 300 to 400 millimeters.

For substances having low vapor pressures at room temperature, diffraction photographs can sometimes be obtained without temperature baths by taking exposures of as much as 20 seconds, especially if the molecule contains several atoms of higher atomic number than carbon. The lowest vapor pressure used in this way was five millimeters. As a rule, clearer patterns result from short exposures with the higher pressures mentioned above. If the temperature required lies between room temperature and 150°C. , the tube supporting the gas nozzle is wound with a heating wire extending from the nozzle down to the vacuum wall. On the outside, the stopcock and specimen holder are enclosed in a cylindrical oven having an opening through which the stopcock is operated. One thermocouple is mounted on the gas nozzle and another in the oven. An ordinary Pyrex stopcock may be used in this temperature range if it is lubricated with a special grease having a low temperature coefficient of viscosity, such as the one made from aluminum stearate and heavy motor oil.⁴ If the substance

⁴ I. E. Puddington, *J. Am. Chem. Soc.*, **65**, 990 (1943).

has enough vapor pressure only at temperatures above 150°C ., it is vaporized from a small heated container placed inside the camera (Fig. 4). This nozzle has no stopcock or valve to control the flow of gas, which accordingly is continuous when the oven is heated. The pressure at room temperature is low enough not to interfere with maintaining the high vacuum in the camera, and the gas entering the camera from the oven is very readily condensed. In any of the nozzle arrangements described, it is important that the electron beam pass directly over the hole through which

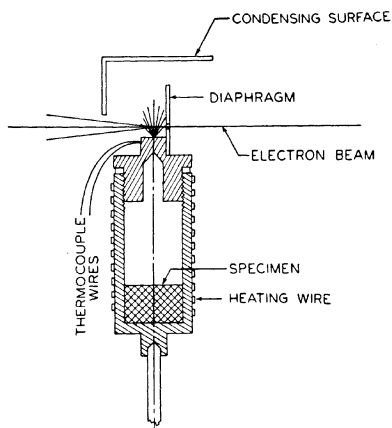


Fig. 4.—Oven for compounds to be vaporized above 150°C .

the gas comes. In each case, the nozzle is supported from a plate which is so mounted on a flexible metal bellows that the nozzle position is adjustable by means of screws on the outside of the camera. A diaphragm with a small hole is mounted on the nozzle so that, when the electron beam passes through the hole, it is correctly aligned with respect to the gas jet.

3. Vacuum System

The principal requirement of the vacuum system is that it be able to maintain the pressure in the camera at 10^{-4} millimeter or less even when the gaseous specimen is introduced. It is necessary, moreover, that there be no direct connection between the electron tube and the diffraction section in order to prevent pressure rises in the electron tube during an exposure; otherwise, destructive arcing may occur in the electron tube.

Multistage high-speed diffusion pumps operating on a low-vapor-pressure oil are connected to the electron tube and to the diffraction section.

The pumps should not be smaller than the four-inch size available with either glass or metal barrels (from *Distillation Products, Inc.*) and rated at about two hundred liters per second. The glass pump does not require water cooling, but above either pump a set of water-cooled baffle plates is required to reduce back-diffusion of pump-oil vapor into the camera. Silicone diffusion pump oil is less susceptible to thermal decomposition or oxidation by air than hydrocarbon oils or esters; but in the pump connected to the gas nozzle (as shown in Fig. 3) where vapors are passed through the pump with each exposure the silicone oil seems to purge itself rather slowly of the absorbed vapor. The use of a cold spot (re-entrant tube cooled with liquid air) in the pump connection over the gas nozzle helps to condense the vapors before they reach the pump.

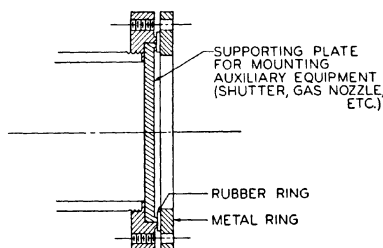


Fig. 5.—Demountable vacuum joint.

The connecting tubes between the pumps and the apparatus must be as short as possible and must be not less than about two inches in diameter in order to take advantage of the high speed of the pumps. On the low-vacuum side, the diffusion pumps may be connected to a single mechanical pump which should have a speed comparable to that of the Cenco Megavac Pump.

The joints between the various sections of the camera and at the various ports where the specimen holder, auxiliary diaphragm supports, and shutter are mounted must be vacuum-tight as well as easily demounted and easily returned to the original positions. Such a joint is shown in figure 5 for a side tube closed by a plate which may be used for mounting any auxiliary device. The plate is recessed into a flange on the tube with as close a mechanical fit as may be desired. The plate and the flange each have a flat-topped bead at the joint between them; on this bead is laid a ring of $\frac{1}{16}$ -inch gasket rubber which is compressed by a metal ring screwed to the flange. The position of the plate is not affected by variable

compression on the gasket, while the joint is easily opened by removing the screws through the metal ring.

Two gages are very useful in operating the vacuum system. One operating in the range from 10^{-1} to 10^{-3} millimeter is indispensable in locating leaks in the system. The most simple and rugged gage for this range is the thermocouple type, in which a pair of wires of dissimilar metals is spot-welded together in the form of an X. A small constant voltage is applied to one pair of dissimilar leads; a microammeter attached to the other pair shows a reading which depends on the temperature of the junction and hence on the gas pressure. When the pressure falls so low that heat loss by gas conduction is much less than the radiation loss, the gage is no longer sensitive. The second gage operates in the range from about 10^{-3} to 10^{-5} millimeter and is connected to the diffraction section where it shows the pressure fluctuations attending the introduction of the sample of vapor. A good type for this use is the modified Knudsen gage described in Strong's book on experimental techniques.⁵ In this gage, a vane is suspended between a pair of heated wires in such a way that gas molecules reaching the vane from the hot wires rotate the vane from its equilibrium position, through an angle which is proportional to the gas pressure. It has the advantage of being continuously indicating, registers both condensable and noncondensable gases, and is not damaged by accidental exposure to high pressure. A second type of gage having these advantages and now available commercially (from the *Radio Corporation of America* or from *Universal X-Ray Products*, Chicago) is the cold cathode ionization gage; application of two or three thousand volts to a cold cathode induces a low electron emission whose effective path to the anode is increased by a strong field from a permanent magnet, the current of positive ions being read on a remote meter. The gage tube and magnet are necessarily mounted near the electron beam of the diffraction camera, and the beam must be shielded from the field of the gage magnet. In a series of successive exposures, either of the high-vacuum gages will indicate when the gas has been sufficiently cleared from the camera before each exposure.

4. Record

The electrons scattered by the gas molecules are recorded on a photographic plate. Because the intensity of the scattered electrons decreases extremely rapidly with increasing scattering angle, the range of intensities to be recorded over the diffraction pattern is usually greater than the photographic emulsion is capable of registering. This difficulty may be partly overcome by taking light and heavy exposures in each series of photographs

⁵ J. Strong, *Procedures in Experimental Physics*. Prentice-Hall, N. Y., 1939, p. 148.

so that the inner and outer parts of the pattern may be studied on different plates. While it should be possible to obtain more precise values of the number of scattered electrons using an electrical method of recording, the full angle range would have to be covered by a long series of measurements throughout which the amount of scattering matter in the gas stream is held constant. The convenience of registering the whole diffraction pattern on a photographic plate in a single exposure of about one second makes the photographic method much more adaptable for extensive use; and, while only approximate values of relative intensities are afforded by the photographic emulsion, they are usually adequate for the determination of inter-nuclear distances.

Photographic plates are preferable to films because they avoid the shrinkage which occurs in the processing of film. In the vacuum of the diffraction camera the supporting layer in the film loses water, and the film shrinks by 2% or more. On subsequent treatment in the photographic solutions the film expands, and shrinks again on drying. Its final size is smaller than originally but larger than in the vacuum-dehydrated state. Since the measured diameters of the interference maxima appearing in the photographic negative are used for determining the size of the scattering molecules, it is important to minimize size changes in the emulsion. If films are used, it is necessary to mark them *inside the vacuum camera* with a known interval of length. With plates, the shrinkage difficulty does not arise, and at the same time the glass backing holds the emulsion flat without any special provision in the plate holder design. The dehydration in the vacuum sometimes causes the emulsion to separate from the backing along the edge of the plate. The emulsion chosen should have considerable latitude because of the great range of intensities to be recorded and will not have high contrast. Eastman Polychrome Plates have been found satisfactory while Process or Lantern Slide Plates are not.

The plate holder must be designed to allow a careful determination of the distance from the photographic plate to the center of the gas nozzle since this camera distance is used with the measurements on the plate in computing scattering angles. The camera in figure 3 provides openings at the specimen section and the plate holder through which a traveling microscope may be focused for measuring the camera distance.

III. INTERPRETATION OF DIFFRACTION PHOTOGRAPHS

1. Theoretical Relation between Intensity of Scattered Electrons, Scattering Angle, and Molecular Structure

The pattern obtained on the photographic plate consists of a series of concentric light and dark rings (Fig. 6). The relative intensities and posi-

tions of these rings must now be interpreted in terms of the structure of the molecules giving rise to the pattern. The theoretical expression desired must apply to the scattering of electrons of uniform energy by a set of molecules having all possible orientations since, in the gas stream, the molecules are randomly related. The motion of the molecules is completely negligible during the time that any one electron is traversing the field of any one molecule. A more complete outline of the derivation described in the following paragraph is given in a review article.⁶

The interaction of the electrons in the beam with a scattering molecule is described by the Schroedinger amplitude equation:

$$\frac{\partial^2 \psi}{\partial x^2} + \frac{\partial^2 \psi}{\partial y^2} + \frac{\partial^2 \psi}{\partial z^2} + \frac{8\pi^2 m}{h^2} (W - V(x, y, z)) \psi = 0 \quad (1)$$

The solution, ψ , is a function of the coordinates, x, y, z , of the electron, and $|\psi|^2$ represents the distribution of electrons in both the incident and scattered beams. W is the energy of the electrons and is a constant determined by the accelerating potential, if the electrons do not lose energy on collision with a molecule. V is the potential energy of an electron in the field of the charges in the molecule (nuclei and electrons). The solution for a stationary molecule is obtained with the aid of the assumptions that the molecule is composed of discrete atoms having spherical distributions of extranuclear charge, that only single scattering of the electrons occurs, and that high-velocity electrons (energies greater than about 20,000 electron volts) will be used.

Fig. 6.—Electron diffraction photograph of carbon tetrachloride.

The resulting expression is averaged over all possible orientations of the stationary molecule, and the formula for the scattered electrons is:

$$I = I_0 (8\pi^2 m e^2 / h^2)^2 (1/r)^2 \sum_i \sum_j f_i f_j (\sin sr_{ij}) / sr_{ij} \quad (2)$$

where I_0 = the intensity of incident electron beam, r = the distance from the scattering center to a point on the photographic plate; $f_i = (Z - F)_i / s^2$, the atomic scattering factor for electrons; Z_i = the atomic number; $F_i = 4\pi \int_0^\infty \rho(r) [(\sin sr) / sr] r^2 dr$, the atomic scattering factor for x-rays⁷.

⁶ L. O. Brockway, *Rev. Modern Phys.*, **8**, 231 (1936).

⁷ R. W. James and G. W. Brindley, *Z. Krist.*, **78**, 370 (1931). L. Pauling and J. Sherman, *ibid.*, **81**, 28 (1932).

$s = 4\pi(\sin \theta/2)/\lambda$; θ = the angle of scattering; λ = the electron wave length; and r_{ij} = the distance between the i th and j th atoms. The double summation extends over all the atoms in the molecule so that the distances between all atom pairs in the molecule appear in the expanded summation. The intensity is then a function of the structure of the molecule and of the scattering angle, θ . With the aid of the above expression, scattering curves (I as a function of θ or of s) can be calculated for any molecular model, and the plausibility of the model tested by comparison of the calculated curve with the photographs.

The formula given above assumes constant interatomic distances, but these distances vary with the thermal motions of the atoms. Since the vapor to be photographed may be at any temperature in the range from 200° to 500° or even 600° K., in many cases the molecules will have vibrational energy, and the vibrational amplitude of the atoms is not always very small in comparison with the interatomic distances. The period of the vibration is much longer than the time an electron spends in the field of the molecule so that, in effect, the electron beam meets a collection of molecules in which the distance between each atom pair varies between a minimum and a maximum value set by the vibrational amplitude. The effect on the scattered electrons may be approximately represented by the factor e^{-as^2} applied to each term in the double summation; a is proportional to the mean square of the vibrational amplitude and is accordingly a function of the temperature. For the terms in which $i = j$ (*i. e.*, scattering by the separate atoms), a is equal to zero; for the others it may reach values up to about 0.010. If the a values for all of the terms were about the same, the effect would be a simple diminution with increasing s . Since, however, the a values are often appreciably larger for some terms than for others, the terms with larger amplitudes are reduced in relative importance. This is illustrated below. The temperature effect in molecules having internal oscillations or restricted rotations requires special treatment.⁸

The total intensity of scattered electrons includes a contribution from those which are scattered with a loss of energy. Because of the energy loss, the wave lengths of these electrons are greater than that of the incident electrons by varying degrees, and there are no coherent relations between the electron waves scattered with loss of energy by different atoms in the same molecule. For this reason, the incoherent scattering is not dependent on the structure of the molecule and is of interest only in connection with quantitative measurements on the scattered electron intensity. The contribution of the incoherent scattering is given by S_i/s^4 for each atom in the molecule, where S_i is a tabulated function⁹ of $0.176sZ_i^{-1/2}$;

⁸ P. Debye, *J. Chem. Phys.*, **9**, 55 (1941).

⁹ L. Bewilogua, *Physik. Z.*, **32**, 740 (1931).

it is very large for small scattering angles but rapidly tends toward zero in a range of s where the coherent scattering is still appreciable.

2. Methods of Comparing the Photographs with the Theoretical Relation

The theoretical relation for the coherent electrons may be written in the form (neglecting the temperature factors):

$$I = A \sum_i \sum_j \frac{(Z - F)_i (Z - F)_j}{s^4} \frac{\sin sr_{ij}}{sr_{ij}} \quad (3)$$

For a simple molecule such as carbon tetrachloride with four equal C—Cl terms and six equal Cl—Cl terms, the expression becomes:

$$I = \frac{A}{s^4} \left[(Z - F)_C^2 + 4(Z - F)_{Cl}^2 + 8(Z - F)_C(Z - F)_{Cl} \frac{\sin sr_{C-Cl}}{sr_{C-Cl}} + 12(Z - F)_{Cl}^2 \frac{\sin sr_{Cl-Cl}}{sr_{Cl-Cl}} \right] \quad (4)$$

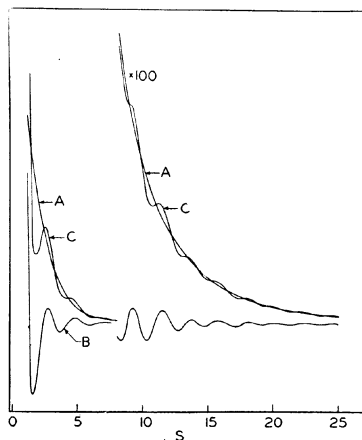


Fig. 7.—Calculated intensity curves for (A) atomic, (B) molecular, and (C) total scattering for carbon tetrachloride.

The terms are of two kinds: the "atomic," due to the separate atoms (summed in Curve A in Fig. 7); and the "molecular," depending on the interatomic distances (Curve B, Fig. 7). In "atomic" terms, \bar{F} functions range smoothly from Z at $s = 0$ to zero at high s values, so that terms of the type $(Z - F)^2/s^4$ start from a high finite value at $s = 0$ and decrease

rapidly without maxima or minima with increasing s . In the "molecular" terms, the damped sine functions produce maxima and minima whose positions and relative amplitudes depend on the interatomic distances and on the chemical elements involved. The amplitudes in the sum of the molecular terms (Curves B) decrease with increasing angle because of the factor, s^{-4} . In the total Curve C (sum of atomic and molecular scattering), the decrease in intensity with increasing angle is so rapid that maxima appear only at 2.5 and 11.0; otherwise there are only inflections about a rapidly falling background. In the application of electron diffraction to the determination of molecular structure, the problem is that of measuring the "molecular" effects in the observed scattering pattern and determining from them the interatomic distances.

The use of microphotometer records of the diffraction pattern in the determination of structure is desirable because of the objective nature of the microphotometer analysis of the pattern. The general form of the

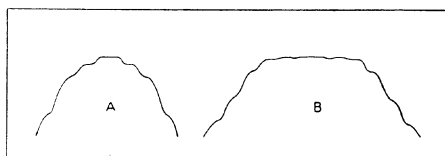


Fig. 8.—Tracings from microphotometer records of carbon tetrachloride photographs, (A) showing the first three apparent maxima and (B) at high sensitivity showing the first five apparent maxima.

scattering curve, *i. e.*, fluctuations about a rapidly falling background, is corroborated by the records of carbon tetrachloride (Fig. 8) taken at a medium (A) and the highest (B) sensitivity setting of the microphotometer. The range of intensities covered from the first to the fifth maximum (visible in B) is 100 to 1 and is too great to be registered at one time. Indeed, the photographic emulsion shows a linear response to electron exposure over a range that is covered by about 3 units in s , or only about two maxima in the carbon tetrachloride pattern. This difficulty may be partially offset by the use of a sector rotated in front of the plate during the exposure (see Section C, page 1133) or by taking a set of graduated exposures. In the latter procedure, several techniques have been tested for increasing the precision of the comparison of the "molecular" part of the intensity in the photometer record and in the calculated curves, but none of them has yet come into general use. The failure of the microphotometer record to register the outer part of the diffraction pattern (beyond the fifth maximum

for carbon tetrachloride), which is observed visually in the photographic negative, has led to the more extensive use of visual methods of interpretation.

The visual appearance of the diffraction photographs (Fig. 6) shows a set of concentric light and dark rings or bands. In negatives of carbon tetrachloride, the dark rings corresponding to the maxima in the molecular curve (B, Fig. 7) have been observed out to the fourteenth. It is evident that the eye does not observe the total intensity (Curve C, Fig. 7) but that it is far more sensitive to fluctuations in intensity, and magnifies the effect of the molecular contributions. The visual effect is not represented directly by Curve B because the apparent intensity of the outermost rings is about 5% of that of the first or second rings while the amplitude of the twelfth maximum in Curve B is only about 0.1% of that of the second. In spite of the fact, however, that the visual appearance cannot be directly correlated with the complete theoretical expression for coherently scattered electrons, the ability of the eye to detect the diffraction pattern far beyond the range of the microphotometer makes the subjective measurements of special value in choosing among scattering curves calculated for different molecular models. A method must then be devised for calculating simplified theoretical curves which may be used in conjunction with visual measurements on the photographic negatives. A rough justification for this correlation based on reactions of the eye has been given.¹⁰

A. VISUAL MEASUREMENTS AND SIMPLIFIED THEORETICAL CURVES

The difference between the visual appearance of the photographs and the complete theoretical curve lies mainly in the much slower decrease in intensity with increasing angle observed visually. In the scattering function (Eq. 4), the rapid fall in intensity is caused by the atomic scattering factor, f , whose denominator contributes a factor of $1/s^4$ in the calculated intensity. Various simplifications of the intensity function have been suggested which will cause the scattering curve to reproduce the appearance of the photographs. The most commonly used simplified curve is obtained by substituting Z , the atomic number, for f . The scattering function for carbon tetrachloride then becomes:

$$I = 8 \times 6 \times 17 \frac{\sin s_{\text{C-Cl}}}{s_{\text{C-Cl}}} + 12 \times 17 \times 17 \frac{\sin s_{\text{Cl-Cl}}}{s_{\text{Cl-Cl}}} \quad (5)$$

The atomic terms have become constant and are of no importance. The curve (I, Fig. 9) does reproduce the visual appearance of the photographs in making the fifth, seventh, and tenth maxima, respectively, stronger than the preceding ones.

¹⁰ L. Pauling and L. O. Brockway, *J. Chem. Phys.*, **2**, 867 (1934).

The agreement between the qualitative features of the negative and the curve calculated for a particular molecular model is a test of the plausibility

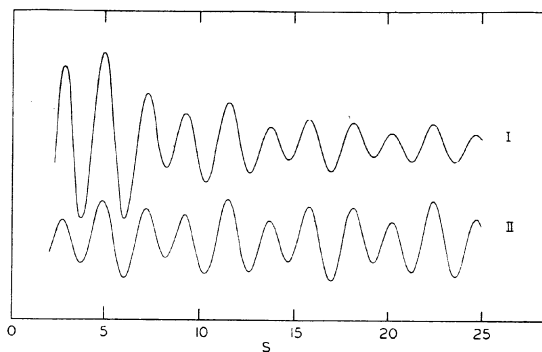


Fig. 9.—Simplified intensity curves for carbon tetrachloride.
(I) $Z_i Z_j (\sin sr_{ij})/sr_{ij}$. (II) $(Z_i Z_j/r_{ij}) \sin sr_{ij}$.

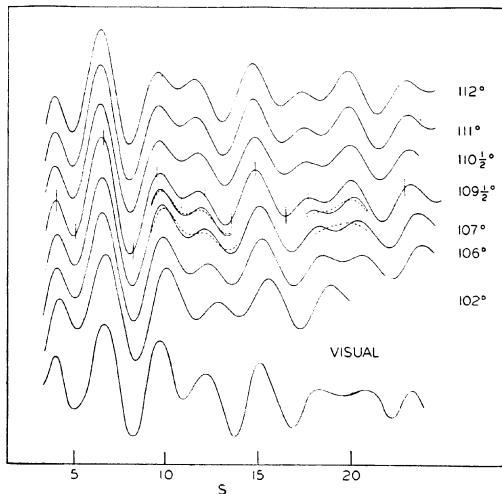


Fig. 10.—Simplified intensity curves of fluoroform showing the effect of variation of FCF angle.

of the model. If distinct reversals of the apparent relative intensities of the rings occur in a given curve, the model for this curve is deemed unsatis-

factory. This comparison of the qualitative features is used in determining the values of the parameters fixing the shape of a molecule. For example, in figure 10 are several intensity curves for fluoroform calculated for models of trigonal symmetry (*i. e.*, having equal FCF angles and equal C—F bond lengths) with FCF angles ranging from 112° down to 102° . The "visual" curve is a representation of the appearance of the photograph. A notable feature is the rough equality of the sixth and seventh peaks with a poorly resolved minimum between. The 112° curve shows the sixth clearly resolved from, and much weaker than, the seventh; with decreasing angle the curves more nearly represent the appearance of the photographs. Below 106° the sixth becomes stronger than the seventh so that this is taken as a lower limit for acceptable values of the angle. The choice of a particular value for the angle is somewhat uncertain because this comparison between a substantially arbitrary intensity function and the subjective appearance of the photograph is by no means a precise process. The function used here does not reproduce the decreasing intensity actually observed toward the outer part of the pattern; and it is therefore possible that the outer of a pair of maxima should be relatively a little higher in this function than it appears in the photograph. On this basis, the angle in fluoroform probably lies between $109\frac{1}{2}^\circ$ and 106° , but a distinction among the values in this range could not be made with any confidence. It may be noted here that the range of uncertainty for a given molecular parameter depends very much on how sensitive the curves are to changes in the model. A similar comparison between the calculated and the visual curves at the third and fourth maxima supports the angle range decided on above. In the curves above $109\frac{1}{2}^\circ$ this pair of peaks is insufficiently resolved, whereas below 106° it is too well separated. In general, qualitative comparisons in different ranges of the acceptable curves must lead to the same conclusions.

If more than one shape parameter (angle or distance ratio) is required to fix the model, the number of intensity curves to be calculated in a complete survey of the structural possibilities rises very rapidly. For each value of the first parameter, curves for several values of the second may be necessary, although it is rarely useful to calculate for two parameters as many as the square of the number of curves considered for a single parameter. The procedure is the same as before; curves for certain pairs of parameter values can be excluded by the qualitative comparison and a range of acceptable parameter values is thereby marked out. For three or more shape parameters, the number of curves to be calculated in a thorough survey becomes impractical and, moreover, several parameter combinations may lead to indistinguishable curves. In the latter case, it is only possible to say that the several models are compatible with the diffraction results,

and a choice among them as well as other possible models not included in the intensity calculations must be based on nondiffraction considerations. For independent determination, two shape parameters is usually the practical upper limit, and the complexity of molecules which can be studied by electron diffraction without recourse to other information is very limited.

It is often useful to limit the variations considered by assumptions based on other experimental results such as dipole moment measurements, spectroscopic measurements, symmetry properties based on chemical reactions, etc., or even on structural theories, provided in any case that the nature of the assumptions is clearly stated. In the fluoroform molecule discussed above, the assumption of trigonal symmetry based on theory and on the spectroscopically observed symmetry in other molecules of the CXY_3 type reduces the number of shape parameters from five to one (when the weakly scattering H atom is neglected or fixed by an assumed C—H distance).

The possible effect on the curves of the exponential temperature factor referred to above must also be considered. In the function, $\exp(-as^2)$, the a value is larger for the terms in the scattering function having the larger amplitudes of vibration, and generally the distances between nonbonded atoms will vary more than those between bonded atoms. The terms for the nonbonded distances will become less important as the scattering angle increases. The damping effect on the nonbonded terms is illustrated for two curves in figure 10. The dotted line on the 107° curve has the F—F term multiplied by the factor, $\exp(-0.002s^2)$; the two segments above correspond to a $108\frac{1}{2}^\circ$ model with the dotted lines having the F—F term multiplied by $\exp(-0.001s^2)$. In the dotted curves the weaker peaks are made still weaker, the effect being larger in the 107° curve where the a value is larger. In this curve the effect is too great because it makes the sixth and seventh peaks coalesce, whereas they are observed as partially resolved. The effect in the $108\frac{1}{2}^\circ$ curve is probably also too large since the sixth peak is reduced to a shelf. Since the magnitude of the coefficients in the temperature factors is usually not known, their effects on the curve cannot be included with any certainty; and some allowance for this uncertainty must be considered in choosing among the models for the best qualitative agreement with the photographs.

The foregoing discussion refers to the apparent relative intensities of the peaks and only in a rough fashion to their positions. Quantitative measurements on the positions not only may afford support for the angles or distance ratios chosen as described but also will fix the scale of the curves and thereby the size of the molecule. In the visual method, a series of points are measured on the photograph and correlated with corresponding points on a calculated curve showing the correct qualitative features. The measurements are made on the diameters of the light and dark rings

on the negative corresponding to minima and maxima in the curves. The negative is mounted on the illuminated translucent screen of a comparator having two carriages fitted with pointers. The points are set at the darkest (or lightest) positions on the ends of a ring diameter; and from the reading of the comparator, the measured distance from gas nozzle to photographic plate and the electron wave length, an experimental value for s [$= 4\pi \sin(\theta/2)/\lambda$] is obtained for the ring measured. With the assumption that each maximum (or minimum) must occur in the photograph and in the calculated curve for the correct model at the same value of sr , the following relation is applied to each of the measurements:

$$(sr)_{\text{exp.}} = (sr)_{\text{theor.}} \quad \text{or} \quad r_{\text{exp.}} = \frac{s}{s_0} r_{\text{theor.}}$$

s_0 is computed from the measured ring diameter, s is read from the curve, $r_{\text{theor.}}$ is the value assumed for any interatomic distance in calculating the theoretical curve, and $r_{\text{exp.}}$ is the experimental result for the distance. A set of $r_{\text{exp.}}$ values for C—Cl in CCl_4 is shown in table I, where the s values in the curve (Eq. 5) were fixed by $r_{\text{theor.}}$ of 1.76 Å. for C—Cl.

TABLE I
EXPERIMENTAL VALUES FOR INTERATOMIC DISTANCES
(CCl_4 -CAMERA DISTANCE = 12.19 CM., $\lambda = 0.0604$ Å.)

Max.	Min.	I_k	s_0	s	C—Cl, Å.
1		20	2.87	2.73	(1.675)
	2	-20	3.78	3.65	(1.697)
2		30	4.91	4.82	(1.727)
	3	-20	6.06	6.04	1.752
3		15	7.14	7.16	1.764
	4	-15	8.22	8.22	1.759
4		10	9.26	9.22	1.751
	5	-10	10.33	10.27	1.752
5		12	11.44	11.48	1.765
	6	-18	12.58	12.64	1.767
6		5	13.65	13.69	1.765
	7	-5	14.71	14.71	1.760
7		5	15.81	15.76	1.756
	8	-2	16.89	16.92	1.764
8		2	18.02	18.10	1.768
	9	-1	19.07	19.18	1.771
9		1	20.14	20.18	1.763
	10	-1	21.22	21.17	1.756
10		1	22.34	22.37	1.762
				AVERAGE:	1.761
				AVERAGE DEVIATION:	0.005

The average deviation of the individual values from the mean value of 1.761 Å. is 0.005 Å., while the maximum deviation is 0.010 Å. and the probable error of the mean is 0.001 Å. The absence of any large deviations

means that the relative positions of the peaks in the photograph are well reproduced by the calculated curve based on the regular tetrahedral model for carbon tetrachloride and supports the probable correctness of this model. (Irregular models are eliminated by the observed zero value for the dipole moment, while the curves for planar models show relative intensities not observed in the photographs.) The first three values for C—Cl in table I are lower than all the others and were not included in the average. This failure of the curve for s values below about 5.0 is always observed. The poor correlation between visual measurements and the curve of equation (5) near the center of the diffraction pattern is related to the very steep fall in the background intensity in this region. While the addition of a falling background would shift the peaks outward in the calculated curve and raise the C—Cl values obtained, the region affected is too small a part of the total pattern to warrant any such attempt.

The excellent agreement among the distance values from all of the peaks beyond the second in carbon tetrachloride is not observed when the pattern has irregular or poorly resolved peaks. Visual measurements on the diameters of a partially resolved pair of rings of equal intensity are too small for the inner and too large for the outer in comparison with the results from the well-resolved, regularly shaped rings in the same pattern. If one of the pair is a weaker subsidiary to the other, the weaker one is shifted in its apparent position away from the stronger one, which may be very little affected. While the occurrence of such irregularities in the pattern is an invaluable aid in choosing among various molecular models, the measurements on the irregular features are not reliable in fixing the scale of the curve, and some allowance must be made in averaging the experimental distance values from the various rings. The usual procedure is to exclude from the average those values based on irregular rings. In figure 10, the positions of the visually measured maxima and minima are indicated in the "visual" curve and marked with vertical lines on the $109\frac{1}{2}^\circ$ curve. Here, the fourth peak and the preceding minimum and the sixth and seventh peaks are marked with broken lines to indicate the expected unreliability of the measurements on their diameters. The seventh peak is included because it was observed to be equal to, and almost unresolved from, the sixth. Considerable deviations are sometimes observed for distinctly resolved peaks because of poor photographs or of difficulty in choosing the model which gives the optimum qualitative agreement with the photographs. These deviations increase the uncertainty of the structural parameters finally reported.

The curves used in the foregoing discussion are based on the function:

$$I = \sum \sum' Z_i Z_j \frac{\sin s r_{ij}}{s r_{ij}} \quad (6)$$

a sum of terms involving $(\sin x)/x$. A table of this function has been published,¹¹ but the calculation is more convenient with the aid of a set of strips tabulating $(\sin sr)/sr$ where each strip runs through a range of s values at intervals of 0.2 and successive strips cover a range of r values at intervals of 0.01. The calculation based on equation (6) consists in choosing the appropriate strips and summing at each s value the tabulated values each multiplied by its proper coefficient.

The availability of calculating aids for summing simple harmonic terms without the damping factor has led to a consideration of the function obtained by multiplying equation (6) through by s :

$$I' = \sum \sum' Z_i Z_j \frac{1}{r_{ij}} \sin sr_{ij} \quad (7)$$

This equation applied to carbon tetrachloride is plotted in figure 9 (Curve II). There is no general decrease of amplitude at larger scattering angles; but the positions of the peaks differ from those in Curve I by less than the plotting error except at low s values, where the comparison with the photographs is unreliable anyway. The interatomic distances obtained matching s values from the curves with observed s_0 values are the same for both curves. Since Curve II also shows the correct qualitative order of intensity at the fifth, seventh, and tenth peaks, such qualitative comparisons can no doubt be used in choosing among molecular models as described for the $(\sin x)/x$ type of function. The structural results for either type of function depend for their validity on comparison with the results of other experimental methods applied to the same compound.

B. RADIAL DISTRIBUTION FUNCTION

The procedure described above for interpreting diffraction photographs involves the assumption of possible models for the molecule and the testing of them by intensity calculations. The radial distribution function, on the other hand, provides information about the molecule without any preliminary assumption. The function is calculated on the basis of the observed relation between the intensity and the angle of electron scattering. Several forms of the function have been used, but only one of them will be described here.

The radial distribution function in its simplest form is written as follows:

$$D(r) = \sum C_k (\sin s_k r)/s_k r \quad (8)$$

¹¹ J. Sherman, *Z. Krist.*, **85**, 404 (1933).

$D(r)$ represents the product of the scattering powers located in volume elements separated by the vector r . C_k depends on the observed intensity at the point s_k . The function in this form can be obtained by applying the Fourier conversion to the theoretical intensity expression in which the distribution of charge responsible for scattering the electrons is treated as a continuous function and not divided into discrete atoms. In this case, the factor C_k is equal to $s^4 I(s)$, and the summation is an integral from zero to infinity over the variable s . The practical use of the distribution function under these conditions is impossible, however, because of the difficulty in obtaining absolute values for the intensity of scattering over a wide range of s values. Since the intensities are observed visually and, therefore, do not represent the actual intensity of scattered electrons correctly for the reasons described in the preceding section, their use in equation (8) introduces an empirical factor which requires that the distribution function depend for its validity on tests with materials of known structure. The simplest procedure is to make visual estimates of the relative intensities for each of the maxima appearing in the diffraction photographs and, with the s values locating these maxima, to use the summation of a finite number of terms represented in equation (8) in place of the integral.

An illustration of this method for carbon tetrachloride is shown in figure 11, in which the coefficients, C_k , are set equal to the estimated relative intensities, I_k , given in table I. It will be noted that terms are included for the visually observed minima with negative coefficients; the resulting curve shows two peaks in the range from 1.0 to 3.5 Å., corresponding to the C—Cl and Cl—Cl distances. It is generally true that $D(r)$ is large when vector r is equal in length to the distance between a pair of atomic nuclei, because these distances are associated with the greatest scattering power. In the case of carbon tetrachloride, the agreement between the position of the peak in the radial distribution function and the values for the interatomic distances given by the use of theoretical intensity curves (as shown by the vertical lines at 1.76 and 2.87 Å.) is very good. When the number of terms used is small, however, spurious peaks often appear at positions not corresponding to actual interatomic distances, and the position of the true peaks may also be somewhat shifted. When the number of maxima in the photographs is small, it is accordingly useful to sketch a "visual" curve representing the appearance of the photograph with the positions of the maxima and minima determined by their observed s values and the relative heights representing their estimated intensities as shown for fluoroform in figure 10. The ordinates of this curve are read at intervals of 0.4 or less, and in this way the number of terms in the summation of equation (8) for a given range of s values is increased, and the summation more nearly approximates to an integral. The prominence of spurious peaks in the distribu-

tion function is reduced and the resolution of close-lying distances is improved, although at the expense of increased labor since the summation may now include from 50 to 100 terms. The distribution function for fluoroform in figure 11 was obtained in this way. The two vertical lines here represent by their lengths the relative scattering powers associated with the CF term at 1.35 Å. and the FF term at 2.20 Å. A useful modification of the coefficient, C_k , has been proposed in which the visually estimated intensity, I_k , is multiplied by $s^2 \exp(-as^2)$, with the effect of increasing the relative importance of the middle part of the observed pattern

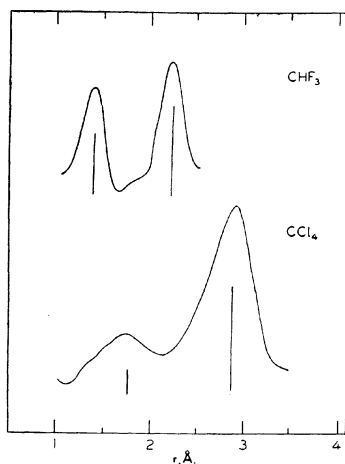


Fig. 11.—Radial distribution curves for fluoroform and carbon tetrachloride. The expected relative intensities and positions of the peaks are shown by the vertical lines.

where the measurements of the s_0 values are more precise. Coefficient a in the exponential is adjusted to make the total coefficient of the last term equal to one-tenth that of the first term.

The usefulness of the radial distribution function lies in the information which it affords about the structure of the molecule without requiring any supplementary knowledge. It is limited by its failure to distinguish between interatomic separations having nearly the same value, and also by the difficulty of allowing suitably for the lesser reliability of poorly defined maxima in the photographs; nevertheless, it helps to exclude whole ranges of structural parameter values in considering molecular models for theoretical intensity calculations. This is especially useful for molecules having

too many parameters to be determined only by the use of intensity curves; and without the distribution function the reliability of the final results reported would, in many cases, be much less.

C. USE OF ROTATING SECTOR

An interesting development by Debye¹² and Hassel¹³ provides an experimental method of compensating for the rapid decrease of intensity of scattered electrons with increasing angle. A brass sector is mounted just in front of the photographic plate and rotated at a speed of 600 r. p. m. or more about a center lying on the electron beam. The sector is cut so that the opening at any radius is proportional to any desired function of s . In the work of Hassel and his collaborators, the sector opening is made proportional to s^2 , which has the effect of multiplying the scattering expression of equation (2) by a factor, s^2 . The new function shows real maxima and minima which can be recorded and measured on a microphotometer; and Hassel¹⁴ has used these data in the calculation of radial distribution functions used for structure determinations without the calculation of intensity curves.

The use of rotating sectors offers the possibility of making quantitative objective measurements on the intensity of scattering of electrons by gases because the scattering at larger angles can be increased in a known way which will bring the intensity at all angles within the range of the photographic emulsion. For the determination of internuclear distances in simple molecules composed of one kind of atom, the sector opening could be made proportional to $1/f^2$, and the recorded electron intensities then would actually be equal to a summation of $(\sin sr)/sr$ terms with constant coefficients. For molecules containing different elements, some kind of average atomic scattering factor could be used for f so that the coefficients of the damped sine terms would not change very rapidly with increasing s . These possibilities have not yet been sufficiently explored and tested, but rotating sectors will come into more general usage in the diffraction study of the molecular structures of gases.

3. Recommended Procedure in Structure Determination

After the photographs have been taken, the ring diameters are measured and the relative intensities estimated. The first step in interpreting the

¹² P. Debye, *Physik. Z.*, **40**, 66, 404 (1939).

¹³ O. Hassel and T. Taarland, *Tids. Kjem. Bergvesen*, **20**, 167 (1940). C. Finbak, O. Hassel, and B. Ottar, *Arch. Math. Naturvidenskab*, **44**, No. 13, 1 (1941). C. Finbak and O. Hassel, *ibid.*, **45**, No. 3, 1 (1941).

¹⁴ H. Viervoll, *Acta Chem. Scand.*, **1**, 120 (1947). O. Hassel and H. Viervoll, *ibid.*, **1**, 149 (1947).

pattern is the calculation of the radial distribution function. While the number of reliable values for interatomic distances afforded by this curve is usually not great enough to fix the structure, it will often fix two or three of the distances associated with the strongest terms in the intensity function and thereby limit the range of parameter values to be included in the molecular models for intensity curves. It is not usually satisfactory to base the structure determination on the radial distribution function alone.

The next step is the choice of models on which to base the intensity calculations. Ordinarily, the general structure of the compound (*i. e.*, the order of attachment of the atoms) may be assumed from the structural formula based on the chemical properties. In a saturated compound of known composition, for example, the possible number of structural arrangements which provide each kind of atom with the correct number of closest neighbors is never large, at least in compounds which are not too complex for treatment by diffraction methods. It is necessary, of course, that the specimen photographed consist of a single isomer if detailed structural information is to be obtained. The principal problem in the choice of models is

TABLE II
ATOMIC RADII FOR ELEMENTS IN ORGANIC COMPOUNDS^a

Element	Atomic radius, Å.	Element	Atomic radius, Å.
Hydrogen	0.32	Silicon	1.17
Carbon	0.77 (single)	Phosphorus	1.10
	0.67 (double)	Sulfur	1.04
	0.60 (triple)	Chlorine	0.99
Nitrogen	0.70 (single)	Germanium	1.22
	0.61 (double)	Arsenic	1.21
	0.55 (triple)	Selenium	1.17
Oxygen	0.66 (single)	Bromine	1.14
	0.56 (double)	Antimony	1.41
	0.51 (triple)	Tellurium	1.37
Fluorine	0.64	Iodine	1.33

^a L. Pauling, *Nature of the Chemical Bond*. Cornell Univ. Press, Ithaca, 1940, p. 164.

deciding on the range of interatomic distances to use. In the case of bonded atom pairs, the problem is simplified by the results of previous investigations. In compounds involving single bonds between carbon atoms, the distances observed for these bonds have never been greater than 1.55 Å. nor smaller than 1.45 Å., even in conjugated systems. The first trial models will accordingly use carbon-carbon single-bond distances in this range, while later models may involve other values in a test of the changes required to cause disagreement between the curves and photographs. In the same way, the distances first tested for carbon-carbon double bonds will lie near 1.34 Å., for carbon-carbon triple bonds near 1.20 Å., and for

bonds in aromatic rings near 1.40 Å. This information for carbon and other nonmetallic elements is summarized in table II. The sum of these radii for the pair of elements in question is a value for the bond distance lying near all previously observed values, and is therefore a value to be tested in intensity calculations. Small deviations from the radius sums are often observed, but deviations as large as 30% would certainly throw the observations under suspicion.

A corresponding guide for plausible nonbonded distances can be given only in terms of the angles between the bonded distances. On a symmetrically substituted atom (in Table II) four bonds will form angles at $109^{\circ}28'$, and on unsymmetrically substituted atoms the angle values will usually not differ from 110° by more than a few degrees. The smallest angle observed is larger than 100° (except for 60° in cyclopropane and about 90° in cyclobutane derivatives). On an unsaturated atom three bonds are coplanar with angles ranging from 110° to 130° ; in aromatic rings the angles are near 120° with the bonds external to the ring also occurring at angles near 120° . Two bonds (two double or a single and a triple) on an atom lie at 180° . For the distance between a pair of atoms separated by two or more atoms, the possibility of rotation or wide oscillations about the intervening bonds must always be considered. This statement of characteristic previous results ought not to limit the range of values tested, except that, in the case of complicated molecules involving too many structural parameters for independent determination, some may be assumed from previous results on similar compounds. In any event, the values indicated should be included among those tested.

After the calculation of several intensity curves, a choice is made by the qualitative comparison between curves and photographs as outlined in the preceding section (pp. 1124–1130). The calculation of additional curves will then be made as required. It should be noted that two models differing only by a constant ratio between all of their corresponding atomic separations will give curves showing identical qualitative features and differing in their scales by the same constant ratio; one curve affords a complete test of all models having the same relative distances. On the curves which are good qualitatively, the positions of the maxima and minima are read (in s units) and tabulated for comparison with the s_0 values computed from the diameters measured on the photographs. The s/s_0 ratios are then averaged (with due allowance for their relative reliability), and the average ratio applied to the distances assumed in the model gives experimental values for the distances.

IV. ACCURACY OF RESULTS AND LIMITATIONS OF THE METHOD

The accuracy of the results of the visual method is affected first by the precision of the s_0 values for the observed rings. The s_0 function depends on the electron wave length, the camera distance from scatterer to plate, and the ring diameters. The wave length is fixed by the accelerating potential; and this can be readily controlled so the uncertainty in the wave length due to fluctuating voltage is as little as 0.2%. The actual value of the wave length as determined by calibration with gold-foil pictures is reliable to about 0.3% or the limiting precision of the line-spacing measurements in the photographs of gold. The camera distance of 10 to 30 centimeters can be measured to about 0.2% with the proper camera design. The ring diameters measured visually with pointers on a comparator mounting are reproducible with about 0.5% maximum deviation for a single observer on sharp, well-defined rings. For different observers the average value of diameter measurements on the same ring may differ by 0.5%. The probable error of the s_0 values in favorable cases is less than 1%; for rings which are not clearly marked in the pattern the variations in the diameter values may amount to several per cent, and such values would not be included in averaging the s/s_0 ratios.

The s values taken from the maxima and minima in a calculated curve may be determined with very high precision by the use of small intervals in the calculation; for a molecule of one parameter (such as a diatomic molecule or the CX_4 regular tetrahedron), the uncertainty in s values can conveniently be kept to a fraction of 1%. The most questionable step in the visual method is the correlation of the calculated curves with the visual appearance of the photograph. By the nature of the procedure it is impossible to make a direct objective test of the reliability of the correlation no matter how satisfying the qualitative agreement between the two patterns may be. A test of the method is afforded, however, by comparing the results of the electron diffraction treatment and of other experimental investigations on the same compounds. For the diatomic molecules, Cl_2 , Br_2 , I_2 , and ICl , the deviations of the electron diffraction values from the more reliable spectroscopic results are +1.1, +0.4, -0.4, and -0.6%, respectively. The electron diffraction value for C—Cl in CCl_4 is 1.76 Å.; the x-ray value¹⁵ is 1.75 Å.—a difference equal to about one-half the experimental error of either method.

The over-all uncertainty in the experimental result is about 1% in the best cases. This figure is an estimated probable error. The formula for

¹⁵ C. Degard, J. Pierard, and W. van der Grinten, *Nature*, **136**, 142 (1935).

probable error as a function of the deviations from the mean in a series of repeated measurements is not applicable here as a measure of the reliability of the experimental results. It is true that several individual values for the interatomic distance are provided by the various rings on a single photograph; and, while the coherence of these values is one of the criteria for acceptability of a particular model, the probable error of the mean value does not show the effect of systematic subjective errors in measuring the plates. The mean s/s_0 ratios determined by different observers often deviate by more than the probable error of the mean of the values from a single observer; and unless a large number of observers always measure each set of plates the experimental uncertainty is larger than the probable error of the mean s/s_0 ratio.

With two or more parameters to be determined, their uncertainties are not uniform but may be very large for some of the parameters and quite small for others. The intensity expression is a sum of terms with various coefficients; and, for a term whose coefficient is small compared with the other coefficients, the corresponding interatomic distance may be changed over a considerable range without producing a detectable effect in the calculated curve. An extreme example is the case of chloroform: the H—Cl and H—C coefficients are small enough compared with those for the Cl—Cl and C—Cl terms that the hydrogen atom cannot be located at all from the diffraction data. The calculated curves are also insensitive to changes in bond angles in the model when the bond angles are near 180° because the distance between the nonbonded atoms changes with the cosine of the bond angle. Since the angles or distance ratios fixing the geometry of the model are determined by comparing the qualitative features of several calculated curves with the photographs, a definite qualitative disagreement must appear in a curve if its parameter values are not to be included in the range reported. In particular, the observed agreement between the photographs and one curve does not fix the parameter in question unless other possible values have been definitely excluded. The exclusion of a curve is questionable unless there is a considerable discrepancy in the relative heights of the peaks in the curve and in the photographs, since it is impossible to make a quantitative intensity correlation between the simplified scattering function and the visually observed peaks.

These factors mean that many molecular structures can be only partially determined by electron diffraction. These include molecules combining atoms of high atomic number with those of low atomic number or containing a number of identical interatomic distances. An illustration of the latter difficulty is found in the paraffin hydrocarbons. In hexane, for example, the five distances equal to the C_1-C_2 separation and the four equal to the C_1-C_3 separation predominate in the scattering function:

and the longer distances in the three weaker terms can be only roughly determined. The longer distances are the only ones which change with rotation around the bonds, and therefore it is difficult, if not impossible, to distinguish among various configurations of the hexane molecule. The distinction between hexane and heptane or between the normal and the branched isomers is also difficult because the differences occur in the weak terms in the scattering function. In molecules containing substituted phenyl groups, it may happen that the orientation of the benzene rings is indeterminate because of the large number of fixed distances which do not depend on the orientation of the rings. In all such cases as those mentioned here, some of the longer distances will remain more or less indeterminate while the shorter distances may be reported with the usual accuracy.

In molecules too complicated for complete determination by electron diffraction, it is often useful to assume the size and shape of certain groups (such as the size and coplanarity of aromatic rings or the bond angles and distances in a CCl_3 group) and to determine by diffraction the remaining parameters. In such a case, it should be borne in mind that the validity of the conclusions depends not only on the diffraction data but also on the original assumptions. A small change in the assumed parameters may have a considerable effect on the diffraction results.

The occurrence of impurities in the diffraction specimen is another possible source of uncertainty in the results. No serious error is caused as long as the relative scattering power of the impurity is only a few per cent of that of the principal material in the gas mixture which reaches the electron beam. Considerable amounts of an impurity of low relative volatility or of low scattering power can be tolerated; but if the impurity contains heavier atoms than the desired material its concentration ought not to exceed a fraction of one per cent. The effect of a suspected impurity can always be tested by combining its electron scattering curve with that of the substance under test in the proportion of their molecular concentrations.

Electron diffraction studies have been made of more than 300 organic molecules, and the results are available on about 275. The number of compounds classified according to the number of carbon atoms per molecule is as follows: one carbon—42; two carbons—94; three carbons—24; four carbons—41; five carbons—17; six carbons, aliphatic—22; six carbons, aromatic—31; twelve carbons—4. The majority of these compounds are hydrocarbons and halogen derivatives. The structural results obtained through June, 1943, have been tabulated.^{6, 16, 17} The diffraction

¹⁶ L. R. Maxwell, *J. Optical Soc. Am.*, **30**, 374 (1940).

¹⁷ G. W. Wheland, *The Theory of Resonance*. Wiley, New York, 1944, Appendix, p. 286.

and spectroscopic results for the simpler hydrocarbons have been discussed in *Annual Reports of the Chemical Society*.¹⁸

One of the interesting structural problems studied by electron diffraction is presented by the organic fluorides. Early measurements showed that the CF bond length in monofluorides is 0.05 or 0.06 Å. longer than in polyfluorides (having two or more fluorine atoms on the same carbon atom). This difference is paralleled by a very great increase in the stability of the polyfluorides toward hydrolysis in comparison with the monofluorides.¹⁹ Subsequent work has indicated that the fluorine atoms influence not only each other but other atoms attached to the carbon. For example, the chlorine atom in CF₃Cl is held closer to the carbon and is removed with much more difficulty than the chlorine atom in CH₃Cl. Also, in polyfluoroethanes, the bond between the carbon atoms is shorter and much more resistant to rupture in oxidizing reactions than in the unsubstituted ethane. In trifluoroacetic acid, the carbon bond is only 1.48 Å., whereas it is 1.54 Å. in acetic acid itself. The possibility of making a quantitative correlation between bond distance and chemical reactivity in standard reactions afforded by the results of diffraction experiments is one of the most important applications of the method.

A recent study²⁰ of the monomeric and dimeric forms of formic and acetic acid shows that, in the carboxyl group, the carbon atom holds the two oxygen atoms with unequal bonds in both the unassociated and associated forms. The longer C—O bond is shortened by about 0.06 Å. on the formation of the dimer, but a difference of about 0.10 Å. between the two C—O bonds persists. The observed nonequivalence of the two C—O bonds in the dimer is evidence for the nonequivalence of the two OH linkages. Other recent investigations have supported the previously observed constancy of the single C—C bond length at or near 1.54 Å., of the double C=C bond at 1.34 Å., of the triple C≡C bond at 1.20 Å.; the observed small interaction of two adjacent single bonds, of adjacent single and double bonds; and the large interaction of adjacent single and triple bonds.

¹⁸ L. O. Brockway, *Ann. Repts. Chem. Soc. (London)*, **34**, 196 (1937).

¹⁹ A. L. Henne, in H. Gilman, *Organic Chemistry*. Wiley, New York, 1943, p. 956.

²⁰ J. Karle and L. O. Brockway, *J. Am. Chem. Soc.*, **66**, 574 (1944).

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REFRACTOMETRY

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I. GENERAL

1. Refractive Index

When light passes from one medium, m , into another medium, M , it undergoes a change in velocity ($v_m \rightarrow v_M$), and unless the beam is perpendicular to the boundary between m and M , also in direction. The change in direction is measured in terms of the angle of incidence, i_m , and the angle of refraction, r_M , with the normal ($N-N'$) to the boundary, as shown in figure 1. Snell's Law of Refraction states that the ratio:

$$n' = \sin i_m / \sin r_M \quad (1)$$

is a constant, where n' is the refractive index of M relative to medium m . According to the wave theory of light, n' is identical with the ratio v_m/v_M . It has become the practice to refer the index of refraction to air, *i. e.*, we define the refractive index n of a single isotropic medium by the ratios:

$$n = \frac{v_{\text{air}}}{v_M} = \frac{\sin i_{\text{air}}}{\sin r_M} \quad (2)$$

Thus n is a dimensionless constant whose value for light of a given wave length is determined by the character and state of substance M and of the reference medium, air. If measurements of n are to be strictly comparable, it is evidently necessary to specify the state of the reference medium, as well as to control other variables which affect the velocity of light in the sample itself. The values of n ordinarily given in the literature refer to air at the temperature and pressure of the measurement or (p. 1196) to air at 20° C. The correction to air at S. T. P. (0° C., 760 mm., dry) is usually of the order of magnitude of 1×10^{-5} and is for most purposes negligible. An error in n of 4×10^{-5} could arise in the comparison of two values measured under extreme natural conditions. For a discussion of the reduction to S. T. P., see Tilton.¹

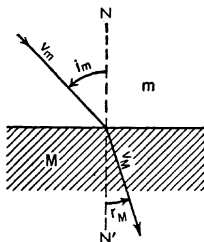


Fig. 1.—Refraction of a light ray.

measured under extreme natural conditions. For a discussion of the reduction to S. T. P., see Tilton.¹

The refraction of gases is usually referred to vacuum as the reference medium. This *absolute index of refraction*, $n_{\text{vac.}}$, is therefore characteristic of a single substance. According to Maxwell's theory, $(n_{\text{vac.}, \infty})^2 = \epsilon\mu$, where ϵ is the dielectric constant, μ is the magnetic permeability of the substance, and ∞ refers to light of infinitely long wave length; μ differs from 1 by less than 10^{-5} for diamagnetic substances, which applies to practically all organic compounds, except free radicals. Actually, the

¹ L. W. Tilton, *J. Research Natl. Bur. Standards*, **14**, 393 (1935).

difference between $n_{vac.}$ and n is very small (about 0.03%). It follows from equation (2) and the definition of $n_{vac.}$ that:

$$n_{vac.} = (n_{vac. \text{ of air}})n \quad (3)$$

For ordinary laboratory conditions, using yellow light:

$$n_{vac.} = 1.00027n \quad (4)$$

In this chapter, anisotropic media are not considered, except in general terms; detailed treatments of birefringence and related phenomena are given in chapters XV and XXIII and in other references. The n values of all crystals except those in the cubic (isotropic) system depend on the direction of transmission and vibration of the light relative to the crystal axes. In general, a single incident light ray will emerge from the crystal as two polarized rays, each corresponding to a different velocity (index) in the crystal, unless the incident ray happens to coincide in direction with one of the optic axes of the crystals. In the latter case, a single ray emerges. The indices along the optic axes and perpendicular to these axes are characteristic of the crystal.^{2, 3} The tetragonal, hexagonal, and rhombohedral crystal systems are uniaxial, *i.e.*, have a single optic axis and two characteristic indices, n_o and n_e . The orthorhombic, monoclinic, and triclinic crystals are biaxial, and have the characteristic indices n_α , n_β , n_γ .^{2, 3} See chapter XV. Therefore in making measurements on solids, it is usually necessary to control the orientation of the specimen. For precautions in preparing and selecting crystals which are homogeneous, see chapter VI. Measurements on specimens which contain occlusions of air or of mother liquor are obviously of little use. Certain flowing liquids also show two indices of refraction, for light polarized parallel and perpendicular to the direction of flow (streaming birefringence). This phenomenon is useful in studying molecular size and shape and is measured by polarimetric methods.⁴

The values of n for organic liquids range from about 1.30 to 1.80, those for organic solids from about 1.3 to 2.5.

The refractive index is of value for the organic chemist in three respects. First, it serves as a means of identification of a substance, as a criterion of its purity, and as a means for the quantitative analysis of its solutions. The usefulness of the refractive index for these purposes is due to the high ac-

² E. M. Chamot and C. W. Mason, *Handbook of Chemical Microscopy*. 2nd ed., Vol. I, Wiley, New York, 1938, p. 290.

³ T. R. P. Gibb, *Optical Methods of Chemical Analysis*. McGraw-Hill, New York, 1942.

⁴ J. T. Edsall, in *Advances in Colloid Science*, E. O. Kraemer, ed., Vol. I. Interscience, New York, 1942, pp. 269-316.

curacy and ease with which it is determined. The precision Abbe refractometer can give n with six significant numbers, while, by interferometric methods, the difference in n between two liquids can be determined with an error only in the eighth decimal place. Second, knowledge of the refractive index is necessary for the evaluation of dipole moments of substances if the dielectric constant is measured at one temperature only (see Chapter XXIV, p. 1611). Third, certain functions of n , *viz.*, the molar refraction, R , are characteristic of a given molecule and indicative of its structure (see page 1162 *et seq.*).

The accurate measurement of refractive index promises to become a common determination in organic chemistry in connection with the estimation of molecular weights of large molecules in solution by their scattering of light (see chapter XXII). An essential datum in this estimation is the difference between the refractive index of the solution, n , and that of the solvent, n_0 . For a 1% solution this difference is about 0.001, so that 1% accuracy in the difference requires n to be accurate in the 5th decimal.

2. Dependence of Refractive Index on Temperature and Pressure

For a wide variety of organic liquids,⁵ an increase in temperature of 1° C. causes a decrease in n of 3.5×10^{-4} to 5.5×10^{-4} . But some liquids, especially at temperatures near the boiling point, have negative temperature coefficients up to 7×10^{-4} . Thus, the commonly used average value of $dn/dt = -4.5 \times 10^{-4}$ may not always be sufficiently accurate. The effect of temperature on n is usually a few per cent greater at the violet end of the spectrum than at the red end. For solids, the values of dn/dt are much less uniform from substance to substance, but as a rule are considerably smaller than for liquids and may be positive or negative. A positive value of dn/dt (relative) may be due either to a positive dn/dt (absolute) or to the effect of the (negative) temperature coefficient (dn_0/dt) of the reference medium (air), since, by differentiating equation (3) and putting $n_a \approx 1$, we have $dn/dt \approx dn/dt - n dn_0/dt$. For many glasses, an increase in temperature causes a small but not negligible increase in n ; *e. g.*, $dn/dt = +1.8 \times 10^{-6}$ per degree for one of the Pulfrich refractometer prisms at room temperature. Usually dn/dt increases slightly with increasing t (roughly, 0.1%/degree C. for solids, 1%/degree C. for liquids).

Thus it is necessary to specify the temperature, t ; this is done by writing $t^\circ \text{C.}$ as a superscript, *e. g.*, n^{25} . Most data on organic compounds are given for 15°, 18°, or 20° C. Ward and Kurtz⁶ recommend the use of 20° C. as

⁵ From the data of J. Timmermans, *J. chim. phys.*, **29**, 529 (1932); **31**, 85 (1934). For empirical rules governing hydrocarbons, see M. R. Lipkin and S. S. Kurtz, Jr. *Ind. Eng. Chem., Anal. Ed.*, **13**, 291 (1941).

⁶ A. L. Ward and S. S. Kurtz, Jr., *Ind. Eng. Chem., Anal. Ed.*, **10**, 559 (1938).

the standard temperature for hydrocarbons. However, in conformity with other physicochemical data, measurements at 25° are becoming increasingly desirable. Even for the roughest measurements for identification purposes, where the accuracy is about ± 0.002 unit of n , the temperature of liquids should be controlled to within about 3°. For an accuracy in n of $\pm 1 \times 10^{-4}$, which is easily attained in rapid routine measurements, the temperature of the sample should be controlled to about $\pm 0.2^\circ$, and corresponding closer for higher accuracy.

For 5th decimal accuracy, or for measurements at extreme temperatures, it is necessary to specify the temperature and pressure of the reference medium (air) surrounding the refractometer (see pp. 1142 and 1195-1196).

For *temperature control* to a few tenths of a degree, one can use tap water which is first passed through an electrically heated or a flame-heated coil, then through the refractometer jacket. Regulation is achieved by changing the rate of flow at the faucet or the rate of heating. The use of thermostats and pumps allows a temperature regulation to within a few thousandths of a degree if the refractometer itself is properly insulated. For information concerning thermostats, see chapter II. Various types of suitable pumps are available commercially.⁷ For pumps which can be built in the laboratory, see Reilly and Rae.⁸

By an increase in pressure of one atmosphere, the refractive index of liquids, according to Tilton,¹ is increased by about 3×10^{-5} . Thus, the normal fluctuations of barometric pressure produce a negligible change in $n_{\text{liq.}}$. The effect on solids should be even smaller.

Various functions of refractive index and density, such as the molar and specific refractions (page 1163) are intended to be independent of temperature and pressure; the empirical formula of Eykmann (page 1169) is the most successful in this respect.

3. Dependence of Refractive Index on Wave Length

The refractive index depends strongly on the wave length of light used. For instance, n^{20} of benzene is 1.49759 when measured with red light of $\lambda = 6563 \text{ \AA.}$, but is 1.52487 with violet light of 4341 \AA. Because of this *optical dispersion*, values of n are comparable only when they refer to the same λ . The kind of light used should be designated by a subscript, as in n_D or n_{5893} . When this is lacking, it may be assumed that the value refers to the sodium D line (5893 \AA.). This, it should be recalled, actually consists of two lines, D_1 (5890 \AA.) and D_2 (5896 \AA.), of nearly equal intensity. The

⁷ American Instrument Co., Silver Spring, Md. Eastern Engineering Co., New Haven, Conn.

⁸ J. Reilly and W. N. Rae, *Physico-chemical Methods*. 3rd ed., Vol. I, Van Nostrand, New York, 1939, p. 239.

difference between n_{D_1} and n_{D_2} can be appreciable for substances of high dispersion; for example, $n_{D_1} - n_{D_2} = 2 \times 10^{-6}$ for benzene. For the effect of the doublet on the critical boundary in the Pulfrich refractometer, see Guild.⁹ With colored materials or in order to determine the optical dispersion, n is measured at other wave lengths, in the past most frequently with the hydrogen lines, H_α (C), H_β (F), and H_γ (G'). The optical dispersion is expressed as $n_{\lambda_1} - n_{\lambda_2}$ or as some simple function of it. Other expressions used are the "nu value," $N = (n_D - 1)/(n_F - n_C)$, and the specific dispersion, $(n_F - n_C)/d$.^{10, 11} With the intense and convenient light sources now available (page 1178), the optical dispersion is readily measured and frequently used. It shows greater differences among closely related compounds than n itself, and has, for instance, been used to determine the concentration of aromatic constituents in hydrocarbon oils.¹¹

DISPERSION FORMULAS

If the relation between n_λ and λ is known,¹² the data, often obtained with helium, mercury, or cadmium lines, can be converted to values at other wave lengths such as those of the sodium or hydrogen spectrum.

In a spectral region which is far from an absorption band, n decreases gradually as λ increases, *i. e.*, we have "normal dispersion." However, within or near a region of absorption, n may pass through a sharp minimum, then, at longer wave lengths, through a sharp maximum. Thus, between the minimum and the maximum there is a region of "anomalous dispersion" in which n will increase with increasing λ . In some cases,^{13, 14} depending on the nature of the absorption band,¹² the n vs. λ curve shows only a flat minimum and maximum, or merely an inflection, in the region of absorption. When the absorption bands are relatively weak, there may be no change detectable in the slope of the n vs. λ curve, using ordinary accuracy, even within the bands.¹³⁻¹⁵ This is the case in the near ultraviolet with many colorless organic liquids for which the principal electronic absorption bands are in the far ultraviolet. Intensely colored substances

⁹ J. Guild, *Proc. Phys. Soc. London*, **30**, 157 (1917-1918).

¹⁰ W. Bielenberg, *Z. angew. Chem.*, **42**, 972 (1929).

¹¹ A. V. Grosse and R. C. Wackher, *Ind. Eng. Chem., Anal. Ed.*, **11**, 614 (1939). A. L. Ward and W. H. Fulweiler, *ibid.*, **6**, 396 (1934). A. L. Ward and S. S. Kurtz, Jr., *ibid.*, **10**, 559 (1938).

¹² F. Eisenlohr, *Spektrochemie der organischen Verbindungen*, Enke, Stuttgart, 1912. See also: S. S. Kurtz, Jr., and A. L. Ward, *J. Franklin Inst.*, **224**, 583, 697 (1937); and K. F. Herzfeld and K. L. Wolf in *Handbuch der Physik*, Vol. XX, Springer, Berlin, 1928, p. 480; H. Kessler, *ibid.*, Vol. XVIII, p. 625.

¹³ T. M. Lowry and C. B. Allsopp, *Proc. Roy. Soc. London*, **A163**, 359 (1937).

¹⁴ K. Feussner, *Z. Physik*, **45**, 689 (1927).

¹⁵ H. Voellmy, *Z. physik. Chem.*, **127**, 305 (1927).

can be expected to show anomalous dispersion in the visible region. Erythrosin¹⁶ shows $n_{\min.} = 1.330$ at $\lambda = 4900 \text{ \AA.}$ and $n_{\max.} = 1.380$ at $\lambda = 5500 \text{ \AA.}$; the absorption maximum comes at $\lambda = 5150 \text{ \AA.}$

In the region of normal dispersion, the *Cauchy formula*, although without theoretical basis, is good enough for rough interpolation:

$$n = A + B/\lambda^2 + C/\lambda^4 \quad (5)$$

where A , B , and C are empirical constants. It is often used in the simplified form:

$$n = A + B/\lambda^2 \quad (6)$$

The constants, A and B , are evaluated from data (n_1, n_2) at two wave lengths, λ_1 and λ_2 :

$$A = (n_1\lambda_1^2 - n_2\lambda_2^2)/(\lambda_1^2 - \lambda_2^2) \quad (7)$$

$$B = (n_1 - A)\lambda_1^2 \quad (8)$$

According to equation (6), the plot of n against $1/\lambda^2$ is a straight line, convenient for graphical interpolation. Values of λ^2 for various spectral lines are given in table VII, page 1179.

The *Hartman interpolation formula*:

$$n - A = B/(\lambda - C)^D \quad (9)$$

also empirical, has similar limitations. The constants, A , B , C , and D , are evaluated from experimental data. In connection with the Hartman formula, tables have been published¹⁷ which facilitate the computation of indices for the C, D, F, and G' lines from observations made on three or more lines of the mercury arc.

In order to obtain an approximate value of the difference, $n_{\lambda_1} - n_{\lambda_2}$, for any pair of λ 's (in \AA.) from measurements of the difference, $n_F - n_C$, one can use expressions (10) and (11), based on the Cauchy formula:

$$(n_{\lambda_1} - n_{\lambda_2})/(n_F - n_C) \approx (1/\lambda_1^2 - 1/\lambda_2^2)/(1/\lambda_F^2 - 1/\lambda_C^2) \quad (10)$$

$$(n_{\lambda_1} - n_{\lambda_2}) \approx \left(\frac{1}{\lambda_1^2} - \frac{1}{\lambda_2^2} \right) \left(\frac{n_F - n_C}{1.9095} \right) \times 10^8 \quad (11)$$

For obtaining approximate absolute values of n_F and n_C from the difference, $n_F - n_C$ and n_D , the empirical rule of Waldmann¹⁸ is useful:

$$(n_D - n_C)/(n_F - n_C) = K = 0.29 \quad (12)$$

¹⁶ B. J. v. d. Plaats, *Ann. Physik*, **47**, 429 (1915).

¹⁷ F. Weigert, in W. Ewald, *Die optische Werkstatt*. Borntraeger, Berlin, 1930 pp. 215-273.

¹⁸ H. Waldmann, *Helv. Chim. Acta*, **21**, 1053 (1938).

This relation holds for a wide variety of organic compounds within about 10%; but with compounds of high dispersion, K is likely to show still greater deviations from the average (0.29).

Equations (6) and (9) are not reliable for extrapolation, *e. g.*, a value of n_C (red) calculated with equation (6) from data on the mercury spectrum (which extends only to the yellow) is likely to be too low. It is preferable to interpolate n_C from data on the helium spectrum, which contains an intense red line at a wave length greater than that of the hydrogen C line.

A more satisfactory expression for the normal dispersion not too close to absorption bands, originating in the classical electromagnetic theory (Drude) and confirmed by quantum theory, has the form:¹²

$$\phi = \frac{n_\lambda^2 - 1}{n_\lambda^2 + 2} = \sum \frac{C_i}{(\nu_0)_i^2 - \nu^2} \quad (13)$$

where C_i is a constant related to transition probabilities, $(\nu_0)_i$ is a "characteristic frequency" related to, but not identical with, the frequency of the maximum light absorption, and ν is the frequency ($\nu = c/\lambda$, where c = velocity of light) of the light used. Concerning an alternative form to the left-hand side of equation (13), see the discussion by Kurtz and Ward.¹⁹ According to equation (13), there will be a term, $C_i/[(\nu_0)_i^2 - \nu^2]$, for each absorption band in the visible and invisible spectrum; but it is often found for colorless substances that only a single term is necessary to represent the change of n with ν in the visible region of the spectrum, even though the substance has more than one absorption band. This means that all but one of the nearby absorption bands are weak, *i. e.*, all nearby C_i values but one are small; or that only one band is near enough to the visible to contribute appreciably to the dispersion in this region, *i. e.*, only one $(\nu_0)_i$ is close enough to ν . Even when several bands contribute to the dispersion, the application of equation (13) to interpolation in the visible and even to extrapolation to long wave lengths, is a fairly simple matter. For small values of ν^2 , far from $(\nu_0)_i$, a plot of $1/\phi$ against ν^2 gives a curve approaching a straight line, no matter how many absorption maxima influence the dispersion.²⁰ Such a linear relation is obvious for the simplest case with only one term.

$$\frac{1}{\phi} = \frac{\nu_0^2}{C} - \frac{1}{C} \nu^2 \quad (14)$$

¹⁹ S. S. Kurtz, Jr., and A. L. Ward, *J. Franklin Inst.*, **222**, 563 (1936); **224**, 583, 697 (1937).

²⁰ P. Wulff, *Z. physik. Chem.*, **B21**, 370 (1933).

Departure from equation (14) may be expected in the region of absorption (*i. e.*, for $\nu \approx \nu_0$) due to the limitations¹² of equation (13).

Experience has shown that a plot of $1/\phi$ vs. ν^2 becomes linear in the visible region of the spectrum for practically all colorless compounds, even though it is certain that these compounds have more than one absorption band in the invisible spectrum. Figure 2, showing the data for liquid benzene obtained by Lowry and Allsopp,²¹ gives a typical dispersion curve, using this "reciprocal plotting method" developed by Wulff.²⁰ The downward curvature at high frequencies is often observed to begin in the blue-violet region

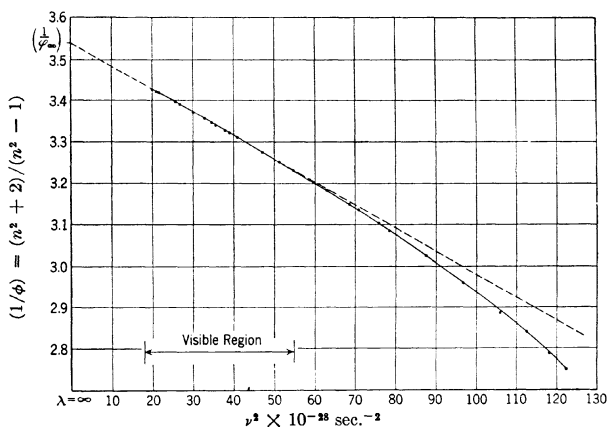


Fig. 2.—Reciprocal plotting method of interpolation and extrapolation of n_λ . Dispersion of liquid benzene at 20° C. from data of Lowry and Allsopp.²¹

of the visible spectrum for colorless compounds of relatively large dispersion; this is to be expected from equation (13), when two or more terms having values of $(\nu_0)_i$ greater than ν influence the dispersion. An upward curvature occasionally observed at the red end of the spectrum indicates that absorption bands in the infrared region are sufficiently near and strong to contribute to the value of $1/\phi$ in the visible region. Interpolation in the linear part of the plot is especially reliable. Values of n_λ can be calculated from the corresponding $(1/\phi)_\lambda$ values by equation (15).

$$n_\lambda^2 = [(1/\phi)_\lambda + 2]/[(1/\phi)_\lambda - 1] \quad (15)$$

The reciprocal plotting method has been applied to the extrapolation of $(1/\phi)_\lambda$ values to infinite wave length.^{20, 22} In this way, the quantity $D =$

²¹ T. M. Lowry and C. B. Allsopp, *Proc. Roy. Soc. London*, **A133**, 26 (1931).

²² N. Bauer and K. Fajans, *J. Am. Chem. Soc.*, **64**, 3023 (1942).

$R_D - R_\infty$ has been obtained from the molar refraction, R , and used as a measure of the molar dispersion.²² In evaluating the electronic polarizability for the calculation of dipole moments (Chapter XXIV, Part II), R_∞ obtained by the above linear extrapolation from measurements in the visible does not include the influence of atomic polarization (absorption bands in the infrared), as long as any upward curvature in the red region is ignored. Other methods of extrapolation, *e. g.*, by the Cauchy formula, are likely to include appreciable contributions from atomic polarization, since the infrared absorption frequently influences the refractive index at the red end of the spectrum.

4. Effect of Concentration on Refractive Index of Solutions

If the refractive index is to be used as a measure of concentration, or vice versa, the most reliable method is interpolation from an empirical calibration curve. In certain cases there exists a linear relationship between these two quantities (see p. 1152), but more generally the data are represented by a curve, the shape of which is influenced by the way in which the concentration is expressed. Molarity, C , is more likely to give a nearly linear relationship than weight per cent, p . For example, the data of Koenig-Gressmann²³ on acetone (n_1)–carbon tetrachloride (n_2) solutions show only a slight departure from the straight line, $C_1 = k(\Delta n)$, where $\Delta n = n - n_2$; $k = 1000 d_1/M_1(n_1 - n_2)$, d_1 = density, M_1 = molecular weight of pure acetone, over the whole range from $C_1 = 0$ to $C_1 = 13.54$ (100% acetone). Thus, for $\Delta n = -0.06481$ (47.46% acetone), the value of C_1 (8.68) calculated from the above linear relation differs from the experimental value (8.75) by only 1%, whereas the plot of p vs. Δn shows a corresponding deviation of 36% from the analogous linear relation, $p_1 = [100/(n_1 - n_2)](\Delta n)$. For solutions in which the components undergo association or in which the intermolecular forces are sufficiently different from those in the pure components, a maximum or minimum in the n vs. C curve may occur. For instance, for water containing 65.61 weight per cent acetone at 25° C., $n = 1.3634$, which is greater than the refractive index of pure water, 1.3326, or of pure acetone, 1.3563.

If it is necessary to predict a value of n for a given concentration or vice versa, without a calibration curve, one can often use equation (16), which is among the simplest of the many functions proposed²⁴⁻²⁷ for relating refractive index, density, d , and composition of a mixture.

²² M. L. Koenig-Gressmann, *thesis*, University of Munich, 1938.

²³ C. Cheneveau, *Ann. chim. phys.*, **12**, 145, 289 (1907).

²⁴ K. Lichtenecker, *Physik. Z.*, **27**, 115 (1926).

²⁵ G. DeLattre, *J. chim. phys.*, **24**, 289 (1927).

²⁶ D. A. G. Bruggeman, *Physik. Z.*, **37**, 906 (1936).

$$\frac{100(n-1)}{d} = \frac{p_1(n_1-1)}{d_1} + \frac{(100-p_1)(n_2-1)}{d_2} \quad (16)$$

The subscripts refer to the two components; p is weight per cent. Equation (16) is based on the assumption that the Gladstone-Dale specific refraction, $r = (n-1)/d$, of a binary mixture is additively composed of the corresponding r values of the components. To solve (16) for n , an experimental value for the density, $d_{\text{exp.}}$, of the solution should be known. For the above-mentioned acetone-water solution, $d_{\text{exp.}} = 0.8809$ and, since $d_1 = 0.7862$ for acetone and $d_2 = 0.9977$ for water, one obtains from equation (16) $n_{\text{calc.}} = 1.3630$ which agrees well with $n_{\text{exp.}} = 1.3634$. It may be possible to estimate d with sufficient accuracy from equation (8) of chapter VI (page 258) if the volume change due to mixing is negligible. However, the formation of the 65.61 weight per cent acetone solution is accompanied by a strong contraction, and the assumption of volume additivity would give $d_{\text{calc.}} = 0.8479$, which is considerably smaller than $d_{\text{exp.}}$; using $d_{\text{calc.}}$ one finds $n_{\text{calc.}} = 1.3494$, which is very much lower than $n_{\text{exp.}}$. A relation based on additivity of the Lorentz-Lorenz refraction (see p. 1170) is often used instead of equation (16). Although the additivity of the Lorentz-Lorenz refraction for mixtures is not always as exact as that of other expressions for refraction, *e. g.*, $n_{\text{calc.}} = 1.3645$ for the above case of 65.61% acetone, using $d_{\text{exp.}}$ in the relation on page 1170, its application has an advantage in that the deviations from additivity are often related in a simple way to the forces within and between the molecules.

Refractivity Intercept.—*Group Analysis.* A useful function in the analysis of complex liquid hydrocarbon mixtures is the refractivity intercept, R_i , of Kurtz and Ward²⁸ which is defined by equation (17), where d is density:

$$R_i = n_i^2 - 0.5d^2 \quad (17)$$

Within a homologous series of hydrocarbons a plot of n^2 vs. d^2 is found empirically to fall on a practically straight line; from one series to another the slope of this line is essentially constant (0.5) but its intercept (R_i) is characteristic of the series. For example, $R_i = 1.0461$ for paraffins and 1.0627 for aromatics. Thus it is possible to estimate the concentration of each of these two classes in a mixture containing no other classes, by assuming additivity of the separate R_i values and solving a pair of linear simultaneous equations. Detailed graphical procedures have been worked out for analyzing a variety of such mixtures, including naphthenes and

²⁸ S. S. Kurtz, Jr., and A. L. Ward, *J. Franklin Inst.*, 222, 563 (1936).

olefins.²⁹⁻³¹ The method requires that the mixture contain several members of each homologous series or none at all, if the composition is completely unknown. Certain compounds, for example, cyclohexane, have anomalous values of R_i . Also, R_i depends somewhat on molecular weight and the choice of "constants" must often be guided by knowledge of the sample, particularly its boiling range.

Another function which has been applied to group analysis of complex mixtures is the Lorentz-Lorenz refraction (page 1157). Grosse³² gives a refractometric method for determining the extent of fluorination of naphthenes, based on the fact that the specific Lorentz-Lorenz refraction is practically a constant for fluoronaphthenes ($r = 0.098$ cc./g.) and for naphthenes ($r = 0.33$ cc./g.), independent of molecular weight, and is considerably different for the two types of compounds. The case of paraffin-fluoroparaffin mixtures is not as simple but can be treated in a similar manner. The method was also used to follow the stage of fluorination of chlorocarbons and should be applicable to other kinds of compounds.

5. Effect of Impurities. Precision Data. Standards

The following formula is useful in certain cases in estimating to a first approximation the degree of purification necessary for measurements of a given accuracy:

$$n - n_0 \approx 0.01p_1(n_1 - n_0) \quad (18)$$

where n , n_0 , and n_1 refer to the mixture, the pure substance, and the impurity, respectively. Equation (18) is based on equation (16) and on the further assumption that the densities of both components are equal to the density of the mixture; it applies best to solutions containing a small amount of solute having physical and chemical properties closely similar to those of the solvent, which is a situation frequently encountered in the final purification of liquids. Other kinds of impurities may have a much larger effect on n than is shown by equation (18). Dissolved air lowers the refractive index of liquids by about 0.01% at saturation. Volume per cent may be used instead of p_1 in equation (18) with about equal success.

One can, and should, estimate the accuracy with which it is justified to measure n for a substance having a probable content p_1 of impurity. For

²⁹ S. S. Kurtz, Jr., and C. E. Headington, *Ind. Eng. Chem., Anal. Ed.*, **9**, 21 (1937).

³⁰ S. S. Kurtz, Jr., I. W. Mills, C. C. Martin, W. T. Harvey, and M. R. Lipkin, *Anal. Chem.*, **19**, 175 (1947).

³¹ R. M. Gooding, N. G. Adams, and H. T. Rall, *Ind. Eng. Chem., Anal. Ed.*, **18**, 2 (1946).

³² A. V. Grosse, Manhattan Project Declassified Document, P.B. 60787, Dec. 9, 1946 (M.D.D.C. 523).

TABLE I
REFERENCE LIQUIDS

Liquid	$t^{\circ}\text{C.}$	n_D^t	$(-dn/dt) \times 10^6$	Literature
Methanol	15	1.3307 ^a	39	(1)
Water	15	1.33339 ^b	07	(13)
	20	1.33299 ^b	09	
	25	1.33250 ^b	11	
	30	1.33194 ^b	12	
Acetone	15	1.3616		(2)
	20	1.3591	50	(3)
Acetic acid	15	1.3739	38	(1)
	25	1.3698		(4)
2,2,4-Trimethylpentane*	20	1.3915		(5)
	25	1.3890		(6), (7)
Methylcyclohexane*	15	1.4256	47	(8)
	20	1.4231		(6)
	25	1.4206		(6)
Chloroform	15	1.4486	59	(8)
Carbon tetrachloride	15	1.4631	55	(8)
	20	1.4603		(9)
	25	1.4576		(3)
Toluene*	15	1.4999	60	(8)
	25	1.4941		
Benzene	15	1.5044	63	(8)
	20	1.5012		(9)
	25	1.4981		(10), (11)
Chlorobenzene	15	1.5275	54	(8)
	20	1.5247		(9)
Dibromomethane	15	1.5446 ^a	55	(12)
Bromobenzene	15	1.5625	49	(8)
Bromoform	15	1.6005	57	(8)
Iodobenzene	15	1.6230 ^a	55	(12)
Carbon disulfide	15	1.6319	78	(2)
Diiodomethane	15	1.7443	64	(12)

* Available as reference standards from *National Bureau of Standards*, Washington, D. C. See page 1155.

^a Helium D, line. ^b Values relative to dry air at $t^{\circ}\text{C.}$ and 760 mm. Hg pressure.

- (1) J. Timmermans and Mme. Hennaut-Roland, *J. chim. phys.*, **27**, 401 (1930).
- (2) J. Timmermans and F. Martin, *J. chim. phys.*, **25**, 411 (1928).
- (3) E. Pahlavorini, *Bull. soc. chim. Belg.*, **36**, 533 (1927).
- (4) N. A. Puschin and P. G. Matavulj, *Z. physik. Chem.*, **A161**, 341 (1932).
- (5) C. P. Smyth and W. N. Stoops, *J. Am. Chem. Soc.*, **50**, 1883 (1928).
- (6) J. P. Wibaut, H. Hoog, S. L. Langedijk, J. Overhoff, and J. Smittenberg, *Rec. trav. chim.*, **58**, 329 (1939).
- (7) D. B. Brooks, F. L. Howard, and H. C. Crafton, Jr., *J. Research Natl. Bur. Standards*, **24**, 33 (1940).
- (8) J. Timmermans and F. Martin, *J. chim. phys.*, **23**, 733 (1926).
- (9) R. M. Davies, *Phil. Mag.*, **21**, 1008 (1936).
- (10) M. Wojciechowski, *J. Research Natl. Bur. Standards*, **17**, 453, 721 (1936).
- (11) M. Wojciechowski, *J. Research Natl. Bur. Standards*, **19**, 347 (1937).
- (12) J. Timmermans and Mme. Hennaut-Roland, *J. chim. phys.*, **29**, 529 (1932).
- (13) L. W. Tilton and J. K. Taylor, *J. Research Natl. Bur. Standards*, **20**, 419 (1938).

example, suppose that a benzene ($n_0^{20} = 1.5010_0$) sample is contaminated with 0.3% (p_1) thiophene ($n_1^{20} = 1.5285_0$). We calculate from equation (18) that $n - n_0 \approx 0.00008$. It is evidently not possible to obtain the n value of benzene to better than about 0.0001 from measurements on a sample which may contain up to 0.3% thiophene. The fact that refractive indices reliable to 0.0001 have been collected for a relatively small number of organic compounds is due mainly to insufficient purity.

The refractometric technique in itself should not be considered as an obstacle to obtaining exact data. For example, it does not require much more labor or skill to measure n'_λ to ± 0.00003 (precision Abbe refractometer) than it does to measure n_D with an accuracy of only ± 0.002 (Fisher refractometer), once the appropriate apparatus has been set up. The relative ease of performing precision measurements is even more striking if differential methods are used. It is actually simpler to measure the difference, $n - n_0 = 0.0006$ to $\pm 5 \times 10^{-7}$ by the precise interferometric technique (p. 1220) than it is to measure this same difference to ± 0.0002 with the Abbe refractometer. However, improper use of the instruments or even defects in their construction (see page 1178) lead to serious errors in refractometry. In order that the technique of measurement can be checked and that the calibration of individual instruments may be verified or corrected, it is advisable to perform tests with standard reference liquids. The importance of comparative differential measurements as a means of obtaining consistent data has been emphasized by Swietoslawski.³³ For differential measurements of refractive index, it is desirable to have a series of reference liquids, the n values of which cover the usual range at intervals of about 0.03 unit. Table I gives the refractive index, n'_λ or n'_D , and the temperature coefficient, $-dn'/dt$, for a number of liquids which have been purified and measured carefully. Most of them were selected from the collection by Timmermans³⁴ of the best values for about 500 organic liquids. The values at 15° C. and the corresponding temperature coefficients were measured at the Bureau International des Etalons in Brussels.

Table II gives the refractive indices of water at 25° C. for various wave lengths corresponding to spectral lines in table VII. The values are taken from the very careful work of Tilton and Taylor,³⁵ who provide an extensive interpolation table by which n'_λ for water can be found to within 1×10^{-4} for any temperature between 0° and 60° (intervals of 0.5°) and for wave

³³ W. Swietoslawski, *J. chim. phys.*, **27**, 329 (1930); *Bull. soc. chim. Mém.*, **49**, 1582 (1931).

³⁴ *Annual Tables of Physical Constants and Numerical Data*. National Research Council, Frick Chem. Lab., Princeton, 1941, Sec. 921C.

³⁵ L. W. Tilton and J. K. Taylor, *J. Research Natl. Bur. Standards*, **20**, 419 (1933).

TABLE II
DISPERSION OF PURE WATER AT 25.00° C. ACCORDING TO TILTON AND TAYLOR^a
(RELATIVE TO DRY AIR AT 25° C. AND 760 MM. HG PRESSURE)

Spectral line	n_D^{25}	Spectral line	n_D^{25}
He ₇₁	1.329544 ₈	Hg ₅	1.333977 ₁
He ₇₂	1.330397 ₈	Hg ₆	1.335859 ₉
H _α	1.330671 ₉	H _β	1.336627 ₈
Na _{8892.6}	1.332502 ₆	H _γ	1.337434 ₇
He ₇₃	1.332555 ₈	H _ε	1.338924 ₈
Hg ₅₇	1.332894 ₁	Hg ₅₇	1.339709 ₈
		Hg ₅₈	1.342238 ₈

^a L. W. Tilton and J. K. Taylor, *J. Research Natl. Bur. Standards*, 20, 419 (1938).

lengths between 4000 and 7000 Å. These data allow the use of water as reference standard in checking the dispersion curves of refractometer prisms. It is to be noted that the difference in n for ordinary water ($n_D^{20} = 1.33300$) and D₂O ($n_D^{20} = 1.32844$) is appreciable.

Samples to be used as standards must, of course, meet rigid specifications. The preparation of standard water is described in chapter VI (page 256). Methods of purification for the other substances in table I are given in the corresponding references. For some of these substances, there is still disagreement between the results of various investigators which may amount to a few units in the 4th decimal. Thus, for highest accuracy, it is imperative, even when the purification is made with great care, to submit reference samples to the *National Bureau of Standards*, Washington D. C., for certification. The Bureau of Standards, under "Test Fee Schedule 445," will measure n_D^{25} at various standard temperatures and wave lengths of both liquids and solids. The testing and calibration of Pulfrich and of Abbe refractometers come under "Test Fee Schedule 444."

The U. S. National Bureau of Standards has made available toluene (Standard Sample 211a), 2,2,4-trimethylpentane (Standard Sample 217), and methylcyclohexane (Standard Sample 218) with certified densities and refractive indices. Table III gives these physical constants at various temperatures and wave lengths for samples of the above standards which were specified to have impurities within the limits 0.04 ± 0.02 , 0.12 ± 0.05 , and 0.36 ± 0.18 mole per cent, respectively. The liquids are sold in units of 5-ml. sealed ampoules at a cost of approximately \$10 each.

According to the certificates, the densities were "measured in a pycnometer of special design having a volume of 108 ml. The temperature of the bath was maintained constant to $\pm 0.01^\circ$ C. It is believed that the uncertainties in the values of density are less than ± 0.00002 g./ml. The values of density are on the basis of weights in vacuum, with the samples at a pressure of one atmosphere and saturated with air. The density of air-free material is greater than that of air-saturated material by about 0.01%. Near one atmosphere, the change of density with pres-

TABLE III
DENSITIES AND REFRACTIVE INDICES CERTIFIED BY THE NATIONAL BUREAU OF STANDARDS

Sample: Temperature: Density, d_t/m_l (air-seal.):		Toluene, 211a			2,2,4-Trimethylpentane, 217			Methylcyclohexane, 218		
		20° C.	25° C.	30° C.	20° C.	25° C.	30° C.	20° C.	25° C.	30° C.
λ , Å.	Line	0.86696	0.86231	0.85764	0.69193	0.68781	0.68366	0.76939	0.76506	0.76077
6678.1	Helium	1.49180	1.48903	1.48619	1.38916	1.38670	1.38424	1.42064	1.41812	1.41560
6562.8	Hydrogen C	1.49243	1.48966	1.48682	1.38945	1.38698	1.38452	1.42094	1.41842	1.41591
5892.6 ^a	Sodium D ₁ , D ₂	1.49693	1.49413	1.49126	1.39145	1.38898	1.38650	1.42312	1.42058	1.41806
5460.7	Mercury e	1.50086	1.49803	1.49514	1.39316	1.39068	1.38820	1.42497	1.42243	1.41989
5015.7	Helium	1.50620	1.50334	1.50041	1.39544	1.39294	1.39044	1.42744	1.42488	1.42233
4861.3	Hydrogen F	1.50847	1.50559	1.50265	1.39639	1.39389	1.39138	1.42847	1.42590	1.42334
4358.3	Mercury g	1.51800	1.51506	1.51206	1.40029	1.39776	1.39523	1.43269	1.43010	1.42752

^a Intensity-weighted mean of doublet, D₁, D₂.

sure is of the order of 0.01% per atmosphere. For a description of the method and apparatus used, reference is made to the following publications: *Technological Paper* No. 77 (1916) and *Bulletin*, 9, 405 (1913)."

"The indices of refraction . . . were measured with a spectrometer by the minimum-deviation method, by use of a water-jacketed hollow prism mounted in a stirred air bath on the spectrometer table. A platinum resistance thermometer was immersed in the liquid during the measurements. The values of refractive index are corrected to refer to air at the listed temperatures and at a pressure of 76 cm. Hg. It is believed that the uncertainties in the values of refractive index are less than ± 0.00002 . As measured on a spectrometer, in air, values of refractive index decrease by 0.000005 for each 1 cm. Hg increase in pressure of the air. See *J. Research Natl. Bur. Standards*, 14, 400 (1935) RP 776. When determined by the use of refractometers, such as those of the Abbe and Pulfrich types, the refractive index is, in general, largely independent of the condition of the air at the emergence face of the refractometer block. This is especially true when a strictly comparative procedure is followed. Consequently, the refractive indices of "unknown" samples thus observed are referred to conditions essentially the same as those for the standard samples with which the instrument is adjusted. See *J. Research Natl. Bur. Standards*, 30, 320 (1943) RP 1535. For a description of the apparatus and method of measurement, reference is made to the following publications: *J. Research Natl. Bur. Standards*, 20, 419 (1938) RP 1085; *J. Optical Soc. Am.*, 32, 371 (1942)."

Approximately 125 other hydrocarbons, including the simpler paraffins, olefins, diolefins, naphthenes, and aromatics have also been made available through the U. S. National Bureau of Standards and the American Petroleum Institute.³⁶ These are not accompanied by refractive index certificates; however, they may be useful as comparison standards in setting up analytical procedures for mixtures of these compounds or in testing the purity of laboratory preparations.

6. Evaluation of Lorentz-Lorenz Molar Refraction

The Lorentz-Lorenz molar refraction, $R = [(n^2 - 1)/(n^2 + 2)]M/d$, is a function of the refractive index, the density, d , and the molecular weight, M , which has been used as an additive and constitutive property (see pp. 1162-1177). It will also be shown that even small deviations from additivity of R allow one to draw important conclusions concerning inter- and intramolecular forces. It is therefore of importance to consider the ways in which exact values of the Lorentz-Lorenz function can be obtained. An extended study of the experimental and theoretical aspects of this problem has been carried out in a series (I-LV) of "Refractometric Investigations."³⁷⁻⁶³ Although this study has been concerned primarily with inorganic substances, the improved apparatus used, in addition to the meth-

³⁶ R. L. Demmerle, *Chem. Eng. News*, 24, 2020 (1946).

³⁷ N. Bauer and K. Fajans (LV), *J. Am. Chem. Soc.*, 64, 3023 (1942).

³⁸ A. Braun and P. Hoelemann (LII), *Z. physik. Chem.*, B34, 357 (1936).

ods of evaluation and theoretical treatment, can be applied to organic substances as well. The papers of the series to which reference will be made throughout this chapter are given in footnotes 37-63.

Pure Liquids.—Two experimental precautions are important in obtaining reliable values of the molar refraction: (1) The measurements of density and of refractive index should both be made on the same sample in order to minimize the influence of impurities. (2) The temperature of measurement of n and of d should not differ appreciably; a direct comparison of the thermometers used is advisable. The temperature difference permissible for a given accuracy can be calculated from the temperature coefficients of n and of d (pp. 1144 and 257, respectively) according to (19).

By differentiating the expression for R , above, one obtains an equation for evaluating the error, ∇R , in the molar refraction caused by a given experimental error, ∇n and ∇d , in n and d , respectively:

$$\frac{\nabla R}{R} = \frac{6n}{(n^2 + 2)(n^2 - 1)} \nabla n - \frac{\nabla d}{d} \quad (19)$$

Consider, for example, the error in R^{25} of ethyl alcohol ($n^{25} = 1.3576$, $d^{25} = 0.7850$) if n is measured at 26.0° C. and d at 25.0° C. Assuming that other errors are negligible and knowing that $-dn/dt = 0.0004$ per degree for this

³⁹ K. Fajans and G. Joos (I), *Z. Physik*, **23**, 1 (1924).

⁴⁰ K. Fajans and C. A. Knorr (II), *Ber.*, **59**, 249 (1926).

⁴¹ K. Fajans, P. Hoelemann, and Z. Shibata (XXI), *Z. physik. Chem.*, **B13**, 354 (1931).

⁴² K. Fajans *et al.* (XXX-XXXVI), *Z. physik. Chem.*, **B24**, 103-214 (1934).

⁴³ W. Geffcken and H. Kohner (X), *Z. physik. Chem.*, **B1**, 456 (1928).

⁴⁴ W. Geffcken (XI), *Z. physik. Chem.*, **B5**, 81 (1929).

⁴⁵ W. Geffcken (XIX), *Z. Elektrochem.*, **37**, 233 (1931).

⁴⁶ W. Geffcken, C. Beckmann, and A. Kruis (XXIII), *Z. physik. Chem.*, **B20**, 398 (1933).

⁴⁷ W. Geffcken and A. Kruis (XXIX), *Z. physik. Chem.*, **B23**, 175 (1933).

⁴⁸ W. Geffcken and A. Kruis, *Z. physik. Chem.*, **B45**, 411 (1939).

⁴⁹ H. Goldschmidt and P. Hoelemann (XLV), *Z. physik. Chem.*, **B32**, 341 (1936).

⁵⁰ P. Hoelemann (XLVI), *Z. physik. Chem.*, **B32**, 353 (1936).

⁵¹ P. Hoelemann and H. Kohner (XLIX), *Z. physik. Chem.*, **B13**, 338 (1931).

⁵² O. Johnson, *thesis*, University of Michigan, 1942.

⁵³ M. L. Koenig-Gressmann, *thesis*, University of Munich, 1938.

⁵⁴ H. Kohner (IX), *Z. physik. Chem.*, **B1**, 427 (1928).

⁵⁵ A. Kruis and W. Geffcken (XXVI), *Z. physik. Chem.*, **A166**, 16 (1933).

⁵⁶ A. Kruis (XLVII, XLVIII, LI), *Z. physik. Chem.*, **B34**, 1, 13, 82 (1936).

⁵⁷ A. Kruis and W. Geffcken (IL, L), *Z. physik. Chem.*, **B34**, 51 and 70 (1936).

⁵⁸ G. Pesce and P. Hoelemann (XXXVIIa), *Z. Elektrochem.*, **40**, 1 (1934).

⁵⁹ P. Wulff (XV), *Z. Krist.*, **77**, 61 (1931).

⁶⁰ P. Wulff and A. Heigl (XVI), *Z. Krist.*, **77**, 107 (1931).

⁶¹ P. Wulff (XXV), *Z. physik. Chem.*, **B21**, 368 (1933).

⁶² P. Wulff and D. Schaller (XXVII), *Z. Krist.*, **A87**, 51 (1934).

⁶³ J. Wüst and H. Reindel (XXXI), *Z. physik. Chem.*, **B24**, 155 (1934).

compound, one substitutes $\nabla n = 4 \times 10^{-4}$ and $\nabla d = 0$ in equation (19), obtaining 0.1% as the error in R^{25} caused by the 1.0° C. temperature difference in the measurement of n and d . A considerably greater error in the absolute value of the temperature, if equal for both measurements, is permissible, since the temperature coefficient of R itself is very small (see page 1177). For instance, if both n and d in the above example were obtained at 26.0° C., the resulting R^{26} value would differ from R^{25} by only about +0.01%, since the temperature coefficient of d is -9×10^{-4} g. per cc. per degree.

Consider also the use of equation (19) in estimating the maximum relative error, $(\nabla R/R)_{\max.}$, in R for ethyl alcohol when the error in both n and d is $\pm 1 \times 10^{-4}$; such an accuracy is easily obtained in routine measurements. Substituting, one has:

$$(\nabla R/R)_{\max.} = \frac{6 \times 1.36}{3.85 \times 0.85} (1 \times 10^{-4}) + \frac{1 \times 10^{-4}}{0.80} = 3.8 \times 10^{-4}$$

Since $R^{25} = 12.78$ cc., the maximum absolute error, $\nabla R_{\max.}$, is 0.005 cc. This accuracy is sufficient for many purposes.

Pure Solids.—The sufficiently accurate direct determination of R for crystals is often difficult and time consuming. Two methods are available which allow an estimation of R of a crystalline substance: (1) Measurements of n (and d) on the molten substance⁶⁴ (see also page 1237). The molar refraction is only slightly influenced by changes in state or temperature (see page 1177); moreover, the small changes in R due to these causes are similar in magnitude for a wide variety of organic compounds, so that a correction can be applied to $R_{\text{liq.}}$. (2) Evaluation of $R_{\text{app.}}$, the apparent molar refraction, of the substance, by measuring the n and d of its solution of a known concentration in a suitable solvent (see page 1160). The refractions of mixtures of organic substances show in general such small deviations from additivity (see page 1170) that the value of $R_{\text{app.}}$ should in most cases be equal to $R_{\text{cryst.}}$ within a few hundredths of a cubic centimeter.

The same experimental precautions and equation (19) for errors apply to solids as to liquids. In the direct determination it is especially important to measure n and d on the same sample, since crystals often contain bubbles or other imperfections. The anisotropy of many solids makes necessary a somewhat different treatment of the data. It has been concluded⁶⁰ that the value of R which in the simplest way represents the average polarizability of a slightly anisotropic solid is obtained from the geometric mean of the characteristic indices of refraction. For biaxial crystals, the mean index \bar{n} is given by:

⁶⁴ M. Furter, *Helv. Chim. Acta*, 21, 1666 (1938).

$$\bar{n} = \sqrt[3]{n_\alpha n_\beta n_\gamma} \quad (20)$$

for uniaxial crystals by:

$$\bar{n} = \sqrt[3]{n_e^2 n_o} \quad (21)$$

For the definition of n_α , n_e , etc., see pages 1143 or 895-902.

Solutions.—The precision technique for measuring the apparent molar refraction of solutes, $R_{\text{app.}}$, has been developed in the series of "Refractometric Investigations" by Kohner,⁵⁴ Geffcken,⁴⁵⁻⁴⁸ and Kruis⁵⁵⁻⁵⁷ for aqueous solutions, by Koenig-Gressmann⁵³ for mixtures of organic liquids and applied by Johnson⁵² to salt solutions in ether and acetone. Geffcken and Kruis emphasize the importance of differential measurements and of a temperature control commensurate with the improved precision. The reliability of the results depends on the following precautions: (1) Solution and solvent should be compared under identical conditions, *e. g.*, at the same temperature and with the same instrument. (2) Measurement of the difference between refractive index of solution and solvent ($n - n_0$) should be made on a sample identical with that used for the determination of the density difference ($d - d_0$). (3) The solvent used in preparing the solution and the reference liquid should come from the same source. (4) Special devices should be employed for transferring and storing liquids to prevent change of concentration of solutions by evaporation. (5) If the change of $R_{\text{app.}}$ with concentration is required, the various concentrations should be made up by diluting a *single* carefully analyzed stock solution, using weight burettes.

The apparent molar refraction, $R_{\text{app.}}$, of a solute of molecular weight M_1 is defined by the condition that the total refraction, R , of a solution containing one mole of solute is the sum of $R_{\text{app.}}$ and the refraction which the amount (W_0 grams) of solvent present in this solution would have in the pure state. We use the Lorentz-Lorenz expression (page 1163) for the specific and molar refraction and designate quantities referring to the solution by no index, those referring to the solvent by the index 0, and those referring to the solute by the index 1. Then it is seen that:

$$R = R_{\text{app.}} + r_0 W_0 \quad (22)$$

and:

$$R_{\text{app.}} = \frac{n^2 - 1}{n^2 + 2} \cdot \frac{M_1 + W_0}{d} - \frac{n_0^2 - 1}{n_0^2 + 2} \cdot \frac{W_0}{d_0} \quad (23)$$

While r_0 is the specific refraction of the solvent in the pure state, $R_{\text{app.}}$ is not identical with the molar refraction of the pure solute, R_1 . The difference, $R_{\text{app.}} - R_1$, indicates the deviation from exact additivity in the mixture due to all possible causes; it includes any refractometric effect of

a change in the state of the solute and the solvent which might arise as a result of mixing. $R_{\text{app.}} - R_1$ will be small for solutions in which the components have similar physical properties, or do not exert strong forces on each other (see pages 1151 and 1170).

When concentrations are expressed in terms of mole fraction, X , we use the formula:

$$X_1 R_{\text{app.}} = \left(\frac{n^2 - 1}{n^2 + 2} \cdot \frac{(X_1 M_1 + X_0 M_0)}{d} \right) - \left(\frac{n_0^2 - 1}{n_0^2 + 2} \cdot \frac{X_0 M_0}{d_0} \right) \quad (24)$$

based on the alternative definition:

$$R = X_1 R_{\text{app.}} + X_0 R_0 \quad (25)$$

In terms of molality, m = moles solute per 1000 grams solvent, $R_{\text{app.}}$ is expressed by:

$$R_{\text{app.}} = \left(\frac{n^2 - 1}{n^2 + 2} \cdot \frac{1}{d} \cdot \frac{1000 + m M_1}{m} \right) - \left(\frac{n_0^2 - 1}{n_0^2 + 2} \cdot \frac{1}{d_0} \cdot \frac{1000}{m} \right) \quad (26)$$

Another very convenient expression for $R_{\text{app.}}$, which separates the influence of n and d on $R_{\text{app.}}$, has been derived by Geffcken:⁴⁴

$$R_{\text{app.}} = \phi(\Phi + \Pi) \quad (27)$$

Π is a function of n :

$$\Pi = \frac{1000}{\phi_0 C} (\phi - \phi_0) \quad (28)$$

where:

$$\phi = (n^2 - 1)/(n^2 + 2) \quad (29)$$

and:

$$\phi_0 = (n_0^2 - 1)/(n_0^2 + 2) \quad (30)$$

Φ , the apparent molar volume of the solute, is a function of d (see chapter VI, page 259):

$$\Phi = \frac{M_1}{d_0} - \frac{1000}{C} \cdot \frac{\Delta d}{d_0} \quad (31)$$

where $\Delta d = d - d_0$ and C = concentration in moles per liter.

The accuracy in $R_{\text{app.}}$ depends primarily on the accuracy of the differences Δn and Δd and of the concentration, C . Kohner⁴⁴ and Johnson⁵² obtained the following approximate formula for the maximum error, $\nabla R_{\text{app.}}$, in $R_{\text{app.}}$ caused by experimental errors, $\nabla(\Delta n)$ and $\nabla(\Delta d)$, in Δn and Δd :

$$\nabla R_{\text{app.}} = \frac{1000}{C} \left[\frac{6n_0}{(n_0^2 + 2)^2} \cdot \nabla(\Delta n) + \frac{\phi_0}{d_0} \cdot \nabla(\Delta d) \right] \quad (32)$$

For the case of dilute aqueous solutions, assuming $0.10 > \Delta n > 0.01$, equation (32) becomes:

$$\nabla R_{\text{app.}} = \frac{100}{C} [6\nabla(\Delta n) + 2\nabla(\Delta d)] \quad (33)$$

If, *e. g.*, $\nabla(\Delta n) = 3 \times 10^{-5}$, $\nabla(\Delta d) = 2 \times 10^{-5}$, and $C = 1$, then $\nabla R_{\text{app.}} = 0.02$ cc./mole. As C becomes smaller, the error in $R_{\text{app.}}$ becomes larger because, with a decrease in Δn and Δd , their constant absolute errors become relatively more important. To obtain a value of $R_{\text{app.}}$ accurate to ± 0.1 cc., one can measure Δn with a Pulfrich refractometer and Δd with a pycnometer (Chapter VI) on solutions as dilute as $0.1 M$. For lower concentrations or higher accuracy, the more sensitive methods directly measuring Δn (interferometer) and Δd (float method,⁴⁶ see chapter VI) should be used.

The error in $R_{\text{app.}}$ caused by an error, ∇p , in the weight (p grams) of solute per 100 grams solution is given by:

$$\nabla R_{\text{app.}} = M_1(r_1 - r_0)(\nabla p/p) \quad (34)$$

where r refers to the specific refraction (refraction per gram of substance). For example, if $(\nabla p/p) = 0.001$ (*i. e.*, per cent error in $p = 0.1\%$) for a solution of *p*-nitrobenzene ($M_1 = 137$, $r_1 = 0.2789$), in benzene ($r_0 = 0.3352$) we have $\nabla R_{\text{app.}} = 137 (0.2789 - 0.3352) (0.001) = -0.008$ cc./mole.

Kohner⁵⁴ has also analyzed the influence of errors in d_0 and n_0 and of impurities.

7. Additivity of Molar Refraction and Its Limitations

A. GENERAL

The molar refraction, R , is a property characteristic of a given molecule and indicative of its structure. For a long time the term "characteristic of the molecule" was understood to mean that the value of R is independent of temperature and pressure and whether the substance is present in the gaseous, liquid, or solid state. In mixtures, R was supposed to constitute the share of the given substance, in proportion to its mole fraction, in the total molar refraction (see Eq. 46, page 1170). "Indicative of the molecular structure" was understood to mean that R of a molecule is a property which can be additively composed of empirical increments, connected with atoms and atomic groups present in the molecule, but also dependent on the constitution, *i. e.*, on the kind of binding between these components.

Thus, the molar refraction has been considered to be at the same time additive and constitutive, a point of view which has been applied to many properties since its introduction by Kopp (1842) in the comparison of boiling points and molar volumes of organic substances (page 259). In the field of molar refraction, the additive method was applied first by Berthelot (1856), using equation (35) for molar

refraction, and by Gladstone and Dale (1863) and Landolt (1864), who applied equation (36). The symbols are explained on page 1157.

$$R = (n^2 - 1) M/d \quad (35)$$

$$R_\lambda = (n_\lambda - 1) M/d \quad (36)$$

In 1882, Landolt based the additive increments on the new Lorentz-Lorenz formula for the specific (Eq. 37) and molar (Eq. 38) refraction for the wave length, λ :

$$r_\lambda = \frac{n_\lambda^2 - 1}{n_\lambda^2 + 2} \cdot \frac{1}{d} \quad (37)$$

$$R_\lambda = \frac{n_\lambda^2 - 1}{n_\lambda^2 + 2} \cdot \frac{M}{d} \quad (38)$$

R and r have the dimension of volume and are expressed in cubic centimeters. Equation (38) is now almost generally used.⁶⁵ It shows that molar refraction depends on wave length λ . In fact, the molar dispersion, $R_\lambda - R_\alpha$, itself is a characteristic property of molecules. Brühl, who since 1880 developed the field considerably, included (1891) in his table of additive constants those for molar dispersion. The

TABLE IV
INCREMENTS FOR THE LORENTZ-LORENZ REFRACTION AND DISPERSION OF ATOMS
AND GROUPS IN CUBIC CENTIMETERS PER MOLE^a

Atom or group	Refraction		Dispersion	
	H _α	D	H _β - H _α	H _γ - H _α
CH ₄	4.598	4.618	0.071	0.113
C	2.413	2.418	0.025	0.056
H	1.092	1.100	0.023	0.029
⌈ (C=C)	1.686	1.733	0.138	0.200
⌈ (C≡C)	2.328	2.398	0.139	0.171
O' (hydroxyl)	1.522	1.525	0.006	0.015
O < (ether)	1.639	1.643	0.012	0.019
O* (carbonyl)	2.189	2.211	0.057	0.078
Cl	5.933	5.967	0.107	0.168
Br	8.803	8.865	0.211	0.340
I	13.757	13.900	0.482	0.775
N (primary amine)	2.309	2.322	0.059	0.086
N (secondary amine)	2.478	2.502	0.086	0.119
N (tertiary amine)	2.808	2.840	0.133	0.186

^a According to F. Eisenlohr, *Z. physik. Chem.*, **75**, 585 (1910); **79**, 129 (1912). See also Landolt-Börnstein, *Physikalisch-Chemische Tabellen*, Vol. II, 986, 1923; *Erg. I*, 527 *et seq.* (1927); *IIb*, 822 *et seq.* (1931); *IIIb*, 1694 *et seq.* (1935).

increments, calculated by Eisenlohr, who revised the values of Brühl, and which are listed in table IV, apply to the sodium D line and to hydrogen lines α , β , and γ . The data given in the following text apply to the D line if wave length is not mentioned.

⁶⁵ See, however, S. S. Kistner, Jr., and A. L. Ward, *J. Franklin Inst.*, **224**, 583. 697 (1937) and M. L. Huggins, *J. Chem. Soc.*, **63**, 116 (1941).

TABLE V

INCREMENTS FOR THE LORENTZ-LORENZ REFRACTION OF BINDING ELECTRONS
AND ELECTRON OCTETS FOR THE D LINE IN CUBIC CENTIMETERS PER MOLE^a

Bond	Equivalent to values in table IV	R_D
C:C:H H	CH ₂ or C + 2 H	4.63
C:H	$\frac{1}{2}$ CH ₄ or $\frac{1}{2}$ C + H	1.7
C:C	$\frac{1}{2}$ C	1.2
C::C	$\overline{\text{I}} + \text{C}$	4.1 _s
C:::C	$\overline{\text{I}} + \frac{1}{2}$ C	6.0
Octet	Equivalent to values in table IV	R_D
C:O:H H	O' + $\frac{1}{2}$ C + H	3.2
C:O:C H	O < + $\frac{2}{3}$ C	2.8 _s
C::O H	O' + $\frac{2}{3}$ C	3.4
C:Cl: H	Cl + $\frac{1}{2}$ C	6.6
C:Br: H	Br + $\frac{1}{2}$ C	9.5
C:I: H	I + $\frac{1}{2}$ C	14.5
C:N:H H	N (primary amine) + $\frac{1}{2}$ C + 2 H	5.1
C:N:C H	N (secondary amine) + $\frac{2}{3}$ C + H	4.8
C:N:C C	N (tertiary amine) + $\frac{2}{3}$ C	4.6 _s

^a According to C. P. Smyth, *Phil. Mag.*, **50**, 361, 715 (1925) and K. Fajans and C. A. Knorr, *Ber.*, **59**, 249 (1926). In the latter paper, the increments of this table were given with four significant figures, as were the refractions in table IV. In view of the discussion on pages 1169 *et seq.*, the R_D values in this table are rounded off and that for CH₂ changed from 4.62 to 4.63. Those in table IV are left unchanged because many calculations found in the literature are based upon them.

The method of constant additive increments has been criticized by Eykman (see page 1169) and others.^{66, 67} In order to apply table IV with the proper discrimination, it is necessary to explain its foundation and to examine its limits. In doing so it is well to take into account the electronic structure of the molecules, since optical properties depend on the state of the valence electrons. We shall consider, besides the increments in table IV, the bond and octet refractions (Table V), which are closely connected with the former. Both sets of constants are based on equation (38).

⁶⁶ See, for example, W. Swietoslowski, *J. Am. Chem. Soc.*, **42**, 1945 (1920).

⁶⁷ J. M. Stevels, *Polarizability and Cohesion Energy, thesis*, Univ. of Leiden, 1937.

B. ATOMIC, BOND, AND OCTET CONSTANTS

The experimental foundation for the application of the principle of additivity is the approximate constancy of the difference between the molar refraction of adjacent members of homologous series, *i. e.*, of the increment R_{CH_2} for the CH_2 group. For liquids at 20° C. and the sodium D line, this value is near 4.6 cc. (see page 1170). The increment, R_{CH_2} , is expressed in the system of atomic refractions by:

$$R_{CH_2} = R_C + 2 R_H \quad (39)$$

and in the system of bond refractions by:

$$R_{CH_2} = R_{C-C} + 2 R_{C-H} \quad (40)$$

Correspondingly, the molar refraction of a saturated hydrocarbon C_nH_{2n+2} is:

$$R = nR_C + (2n + 2)R_H = nR_{CH_2} + 2 R_H \quad (41)$$

or:

$$R = (n - 1)R_{C-C} + (2n + 2)R_{C-H} = (n - 1)R_{CH_2} + 4 R_{C-H} \quad (42)$$

In case of exact additivity of the molar refraction, it would be sufficient to use the experimental value, R , for any saturated hydrocarbon, and the value $R_{CH_2} = 4.6$ in order to obtain the values R_H and R_C or R_{C-C} and R_{C-H} . The relation between the atomic and bond constants is given by equations (43) and (44):⁶⁸

$$R_{C-H} = 1/4 R_C + R_H \quad (43)$$

$$R_{C-C} = 1/2 R_C \quad (44)$$

Similar relations between other bond or octet increments and the corresponding atomic increments, with both sets based on the assumption of additivity, are given in table V. If the additivity is not fulfilled for one system of increments it cannot be fulfilled for the other, if both systems are based on the same experimental data.

The contrary conclusion⁶⁹ that in organosilicon compounds the bond refractions show better additivity than the atomic refractions is due to the following inconsistency. For the derivation of the atomic refraction of silicon the increments used for C, O, H, and halogen atoms were those of table IV, based on compounds not containing silicon. For the derivation of the increments of the bonds Si—C, Si—O, etc., data on organosilicon compounds had to be used.

The fact that table IV contains only one value for C and one for H, both given to three decimals, would seem to imply that the molar refraction of saturated hydrocarbons is additive with corresponding accuracy. This is not the case (see page 1168). Moreover, Brühl found that olefins and acetylenes have considerably larger molar refractions than one would expect on the basis of the atomic constants for C and H. He accounted for this by introducing constant increments for the double, \equiv , and triple, \equiv , carbon to carbon bond. This is not consistent with the fact that, in the atomic system, no increment is used for the single bond. For oxygen and nitrogen, the optical effect caused by a variation in the kind of binding is expressed

⁶⁸ See A. L. von Steiger, *Ber.*, 54, 1381 (1921), and reference 66.

⁶⁹ E. L. Warrick, *J. Am. Chem. Soc.*, 68, 2455 (1946).

differently. Landolt used only one constant for oxygen. Brühl introduced a special atomic constant for carbonyl oxygen (O''), and Conrady (1889) distinguished between etheric ($O<$) and hydroxylic (O') oxygen. This choice of constants is arbitrary; one might just as well use only one atomic constant for oxygen, add an increment for $C=O$, and use different atomic constants of hydrogen depending on whether it is bonded to carbon or oxygen. A corresponding remark applies to the distinction between the three atomic constants of nitrogen in primary, secondary, and tertiary amines. In the first two, some of the hydrogen atoms are bonded to carbon and some to nitrogen, and it is arbitrary to ascribe the optical differences only to the nitrogen.

The above criticism should not impair the practical use of table IV as long as the number and nature of the constants chosen are adequate and the conclusions drawn do not go beyond the limitations of the system. Valuable applications of table IV have been described often,⁷⁰⁻⁷² and need not be discussed here.

Table V was derived in an attempt to understand refractometric data in terms of Lewis' electronic interpretation of Kekulé's valence bonds. A single bond corresponds to two electrons, a double bond to four and a triple bond to six binding electrons. In the case of saturated and unsaturated hydrocarbons in which all electrons are assumed to be used in bonds, it is possible to arrive at values not only for $C:H$ and $C:C$ (see page 1165), but also for $C::C$ and $C:::C$. Thus, the inconsistency of the older system is avoided. However, the values for the double and triple bond are derived on the arbitrary assumption that the refractometric value of $C:H$ in unsaturated hydrocarbons is the same as in saturated. The results obtained were clarifying in several respects. For instance, corresponding to the increased number of electrons involved, the bond refractions increase in the series $C:C$ (1.2), $C::C$ (4.15), and $C:::C$ (6.0). That the increase, from the single to the double and triple bond, is very much larger than proportional to the number of electrons seems to indicate that the latter are "looser" in the unsaturated compounds. This is in agreement with the fact that the energies of dissociation of the double and triple bonds,⁷³ approximately $E_{C=C} = 2E_{C-C} - 20$ kcal. and $E_{C\equiv C} = 3E_{C-C} - 50$ kcal., respectively, are less than proportional to the number of electrons.

In molecules containing other elements besides C and H, only part of the electrons participate in the binding, according to Lewis' theory. Thus, subtracting



from the molar refraction of methyl chloride, $H:C:Cl:$, the refraction of the $C:H$

electrons, assumed to be nearly equal to their refraction in hydrocarbons, one obtains the value of the chlorine octet, two of the electrons of which are supposed to be shared with carbon and six to be unshared. The value obtained (6.6) gives an

⁷⁰ S. Glasstone, *Text-Book of Physical Chemistry*. Van Nostrand, New York, 1940, pp. 518-533.

⁷¹ F. Eisenlohr, *Spektrochemie der organischen Verbindungen*. Enke, Stuttgart, 1912.

⁷² W. Hüchel, *Theoretische Grundlagen der organischen Chemie*. Vol. II, Akadem. Verlagsgesellschaft, Leipzig, 1931, pp. 91-114.

⁷³ K. Fajans, *Ber.*, **55**, 2826 (1922).

indication of the state of the electronic shell of the chlorine octet attached to carbon. Comparison of this value with that of the chlorine octet in the free state (Cl^- , 9.0) and in HCl (6.67) shows that the combination with carbon influences the chlorine octet nearly as strongly as when its electronic shell is penetrated by a proton. In a similar way, it was possible to compare the influence of a proton with that of carbon on fluorine, bromine, or iodine octets, and, in the case of the oxygen octet, the influence of two protons, in H_2O , with that of one proton and one carbon atom, in H_2COH , and with that of two carbons, in H_2COCH_3 .

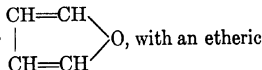
C. LACK OF EXACT ADDITIVITY

It is of theoretical as well as practical importance to gauge how well the additive atomic, bond, or octet constants express the experimental data. The impression is easily gained that the agreement between calculated and observed values is satisfactory except for substances containing either conjugated systems or rings. For instance, for *n*-heptane, $R_{\text{calc.}} = 34.526$ and $R_{\text{obs.}} = 34.54$, and for acetic acid, $R_{\text{calc.}} = 12.972$ and $R_{\text{obs.}} = 12.99$.

Influence of Conjugation.—For a compound, C_6H_{10} , with two double bonds, $R_{\text{calc.}}$ is 28.974. While $R_{\text{obs.}}$, 28.99, of diallyl, $\text{CH}_2=\text{CHCH}_2\text{CH}_2\text{CH}=\text{CH}_2$, approaches this value very closely, $R_{\text{obs.}}$ of 2,4-hexadiene, $\text{CH}_3\text{CH}=\text{CHCH}=\text{CHCH}_3$, is 30.64. The positive deviation (+1.67), exaltation, has been connected with the conjugation of the double bonds in the latter isomer. However, it is not possible to use a new constant increment for the conjugation because, for example, the refraction, 29.75, of $\text{CH}_2=\text{C}(\text{CH}_3)\text{C}(\text{CH}_3)=\text{CH}_2$, the conjugated diisopropenyl, differs less from that of the unconjugated isomer diallyl (by +0.76) than from that of 2,4-hexadiene (by -0.89).

The exaltations have been extensively studied by Brühl, von Auwers and others,⁷⁴ and are considered as anomalous effects. However, exaltations are not restricted to conjugated double bonds. For instance, in deriving the atomic refraction of chlorine, Eisenlohr confirmed the observation of Brühl that a higher value is obtained from acid chlorides (6.336 as an average of five values varying from 6.231 to 6.500) than from other compounds (average 5.967). Eisenlohr assumed that this exaltation in the acid chlorides is due to some kind of conjugation between the carbonyl group and the chlorine atom, and excluded this "special" value of Cl from his table of "normal" atomic refractions.

Influence of Ring Structure.—The opposite effect was observed with furan and analogous substances. The calculated value for



oxygen and two double bonds, is 19.181. Since the double bonds are conjugated, a higher refraction was expected by Brühl. However, the experimental value, 18.426,⁷⁵ is even lower than the calculated. Explanation of this depression as caused by the ring structure did not lead to a general rule. In spite of the ring structure of cyclohexane, $R_{\text{calc.}} = 6 \times 4.618 = 27.708$ agrees well with $R_{\text{obs.}}$

⁷⁴ See, for example, K. von Auwers, *Ann.*, **437**, 63 (1924).

⁷⁵ See E. C. Hughes and J. R. Johnson, *J. Am. Chem. Soc.*, **53**, 737 (1931).

(=27.68) for 15°. Neither can the depression in furan, thiophene, or pyrrole⁷⁶ be attributed to their heterocyclic character. The experimental value of benzene, 26.14 (15°), also shows a depression (0.17) when compared with that calculated for six CH and three double bonds, even if the conjugation is neglected. Recently⁷⁷ resonance has been mentioned in connection with these depression effects.

Differences between Chain Isomers.—While these difficulties have been generally realized for a long time, it is necessary to emphasize that the exact measurements of the last two decades have shown deviations from additivity in many cases which were usually regarded as normal. A good agreement between $R_{\text{calc.}}$ and $R_{\text{obs.}}$ such as that for *n*-heptane is not general even for paraffins. Smyth and Stoops⁷⁸ found, for nine isomeric heptanes at 20°, values between 34.61, for 2,2-dimethylpentane, and 34.25, for 3-ethylpentane. The difference (0.36) between these extremes is three times larger than that (0.118) between (O<) and (O'). Measurements of Timmermans and his associates⁷⁹ contain many examples of chain isomers of substituted saturated hydrocarbons with similar differences in molar refraction. For instance, R for *n*- and *sec*-butyl iodide is 33.48 and 33.85, respectively. Still larger discrepancies are shown by organometallic compounds. The molar refraction of $\text{C}_{12}\text{H}_{28}\text{Pb}$ has the values 78.07, 78.60, and 79.87 for the dimethyldiisomyl, the tetra-*n*-propyl and the tetraisopropyl lead, respectively. If one attempts to derive the atomic refraction of lead from the PbR_4 compounds, one finds 17.31 when R is CH_3 and 20.05 when R is *iso*- C_3H_7 . The corresponding values of the atomic dispersion $H_\gamma - H_\alpha$ are 1.43 and 2.31, respectively.⁸⁰ It would not be justified to average such widely diverging values.

Influence of Association.—Not only isomerism but even mere association of neutral molecules can lead to large changes of molar refraction. While $R_{\text{obs.}} = 12.99$ for liquid acetic acid agrees satisfactorily with $R_{\text{calc.}}$ (see page 1167), the vapor between 190° and 300° C., the range within which acetic acid consists practically completely of the monomer, CH_3COOH , gives the value 13.216 ± 0.002 cc.⁴⁹ For the dimer, $\frac{1}{2}(\text{CH}_3\text{COOH})_2$, the value 13.416 ± 0.01 cc. was derived from measurements between 120° and 190°. Both values for the vapor apply to the Hg_g line; the corresponding value for the liquid is 13.05. It is noteworthy that, while one would be inclined to assume that the dimer is intermediate between the monomer and the liquid, $R_{\text{obs.}}$ of the dimer is higher than that of either.

D. CRITICISM OF METHOD OF CONSTANT INCREMENTS

A detailed analysis even of the experimental material available in 1910 shows that the values for a given atom or type of binding, listed in table IV with four significant figures, are averages of single values which deviate considerably from

⁷⁶ See R. Nasini *et al.*, *Atti accad. Lincei*, **1**, 617 (1886); *Z. physik. Chem.*, **17**, 539 (1895).

⁷⁷ See J. A. Leermakers and A. Weissberger, in H. Gilman, *Organic Chemistry*. 2nd ed., Wiley, New York, 1943, p. 1752.

⁷⁸ C. P. Smyth and W. N. Stoops, *J. Am. Chem. Soc.*, **50**, 1883 (1928).

⁷⁹ J. Timmermans *et al.*, eight papers in *J. chim. phys.*, **23–34** (1926–1937). See references to table I, page 1153.

⁸⁰ G. Grüttner and E. Krause, *Ann.*, **415**, 338 (1918).

each other. For example, in the derivation of the increment $f'' = 1.733$, Eisenlohr excluded not only compounds with conjugated double bonds but also allyl derivatives giving f'' values which he considered much too low. In the remaining 26 compounds, the increments for CH_2 , H, and the different kinds of oxygen, were treated as constants and f'' values were obtained which vary between 1.530 and 1.943. This variation exceeds considerably the experimental error. The average, 1.733, is more or less accidental, and it would be more appropriate to draw the conclusion that $f'' = 1.7 \pm 0.2$. Then it would not be surprising to find⁸¹ that the molar refraction of $\text{CH}_3\text{CH}=\text{CHCH}_2\text{COOH}$ leads to the high value, 1.969, for f'' although the $\text{C}=\text{C}$ bond in this acid is not conjugated with the $\text{C}=\text{O}$ bond of the carbonyl group.

Table IV is almost always used in spite of all these discrepancies. When values calculated from it are compared with experimental molar refractions, the deviations are sometimes very large. For instance, in a series of 26 furan derivatives, the molar exaltation varies from -1.078 for 3-bromofuran to $+5.524$ for furfural pinacolone.⁷⁵ With compounds of an element like fluorine, which is not contained in table IV, one subtracts from the experimental value the sum of all the other increments in order to obtain the atomic refraction of fluorine. In this way, extended investigations⁸² gave for R_F values between 0.68, in $\text{CCl}_2=\text{CClF}$, and 1.60, in $\text{CCl}_2=\text{CCl}-\text{CCl}_2\text{F}$.⁸³ Without discussing the reason for these deviations, one can say only that R_F is around 1 cc.

In the opinion of the writer, Kopp's method of using a limited number of constant increments has been shown by modern measurements of any property of compounds to be only a first approximation. Ostwald⁸⁴ recognized the reason for this lack of strict additivity: a given substitution in two different molecules cannot be expected to have an identical effect. Eykman,⁸⁵ on the basis of very broad experimental material and using for the molar refraction the expression $R = (M/d) \cdot (\eta^2 - 1)/(\eta^2 + 0.4)$, concluded (1893-1911) that the same effect is produced by CH_2 in different types of compounds only in the case of its addition to a long $(\text{CH}_2)_n$ chain. However, in short chains, in branched chains, and in the neighborhood of groups other than CH_2 deviations from additivity occur.

E. CASES OF SATISFACTORY ADDITIVITY OF REFRACTION

A review of modern experimental results also shows satisfactory additivity in homologous series for the Lorentz-Lorenz expression, and with the same limita-

⁸¹ E. Schjånberg, *Z. physik. Chem.*, **A178**, 274 (1937).

⁸² F. Swarts, *J. chim. phys.*, **20**, 30 (1923). A. L. Henne *et al.*, *J. Am. Chem. Soc.*, **1934-1944**.

⁸³ G. Schieman, *Z. physik. Chem.*, **156**, 397 (1931), obtained R_F from 20 compounds: 2.03 was the highest value of the "atomic refraction" of fluorine (in *o*-fluoronitrobenzene); 0.63 was the lowest value (in *p*-fluoroanisole); Schieman derives an average of 0.997. See also A. V. Grosse, R. C. Wackher, and C. B. Linn, *J. Phys. Chem.*, **44**, 275 (1940).

⁸⁴ W. Ostwald, *Grundriss der allgemeinen Chemie*, 3rd ed., Engelmann, Leipzig, 1899, p. 130.

⁸⁵ J. F. Eykman, "Recherches réfractométriques," edited by A. F. Holleman, *Natuurkund. Verhand. Hollandsche Mij. Wetenschappen Haarlem*, **1919**.

tions Eykman found. (See above.) The increment for the CH_2 group within a normal chain, in different series of liquid substances at 20°C ., differs from an average value of 4.63 for the D line only by a few hundredths of a cc.,⁸⁶ except for the first few members of a series, and for series in which a distinct alternation of the increments for successive methylene groups occurs (see, for instance, section F, c, d, f, and g). The value 4.618 given in table IV nearly coincides with this average.

It is therefore recommended in "computing" molar refractions that the experimental values of the nearest homologous substances be used wherever possible and 4.63 for CH_2 . It may be shown by one example that this method is more reliable than the use of the other additive constants of table IV. The molar refraction of the esters of furoic acid, $\text{C}_6\text{H}_5\text{OCOOR}$, would be expected to be, on the basis of table IV, 30.071 for $\text{R} = \text{CH}_3$ and 39.307 for $\text{R} = \text{C}_3\text{H}_7$. When calculated from the experimental value for furan (18.426), the expected values would be 29.310 and 38.546, respectively. The true values⁷⁵ for the methyl ester ($R_{\text{exp.}} = 30.896$) and propyl ester ($R_{\text{exp.}} = 40.255$) differ widely from both sets of the predicted ones. However, if we start with the true value for the ethyl furoate ($R_{\text{exp.}} = 35.61$) and use the increment for CH_2 (4.63), the computed value for the propyl ester ($R_{\text{calc.}} = 40.24$) differs only by 0.015 cc. from the true one. For the first member of the series, the methyl ester, $R_{\text{calc.}} = 35.61 - 4.63 = 30.98$, deviates by 0.08 from $R_{\text{exp.}} = 30.896$.

The Lorentz-Lorenz refraction also shows a satisfactory additivity for mixtures of neutral molecules which do not exert strong forces on each other.^{87, 88} If in a binary mixture x_1 is the mole fraction of component 1, the refraction of one mole of the mixture is represented by formula (45) and the additive value is given by formula (46):

$$R_{\text{exp.}} = \frac{n^2 - 1}{n^2 + 2} \cdot \frac{x_1 M_1 + (1 - x_1) M_2}{d} \quad (45)$$

$$R_{\text{add.}} = x_1 \frac{n_1^2 - 1}{n_1^2 + 2} \cdot \frac{M_1}{d_1} + (1 - x_1) \frac{n_2^2 - 1}{n_2^2 + 2} \cdot \frac{M_2}{d_2} \quad (46)$$

In mixtures of CCl_4 with C_6H_6 , of acetone with CCl_4 or C_6H_6 , the deviations from additivity of the volume of one mole are small (below 0.1 cc. per mole of mixture). Correspondingly, $R_{\text{exp.}} - R_{\text{add.}}$ is small (below 0.06 cc.).

A somewhat wider departure from additivity is shown by measurements of Hubbard⁸⁹ on two mixtures containing CS_2 which is an easily polarizable molecule (see page 1171).

⁸⁶ See A. F. Shepard, A. L. Henne, and T. Midgley, Jr., *J. Am. Chem. Soc.*, **53**, 1948 (1931) (paraffins); P. Bruylants and R. Merckx, *Bull. classe Sci., Acad. roy. Belg.*, **19**, 1003 (1933) (nitriles); P. Ceuterick, *Bull. soc. chim. Belg.*, **45**, 545 (1936) (ketones); H. I. Waterman and W. J. C. De Kok, *Rec. trav. chim.*, **52**, 251, 298 (1933) and **53**, 725 (1934) (olefins).

⁸⁷ See, for example, C. P. Smyth, E. W. Engel, and E. Bright Wilson, *J. Am. Chem. Soc.*, **51**, 1736 (1929), and M. L. Koenig-Gressmann, *thesis*, University of Munich, 1938.

⁸⁸ J. Wellm, *thesis*, University of Königsberg, 1934.

⁸⁹ J. C. Hubbard, *Phys. Rev.*, **1**, **30**, 740 (1910); *Z. physik. Chem.*, **74**, 207 (1910).

F. DEVIATIONS FROM ADDITIVITY CAUSED BY ELECTRIC INTERACTIONS

If one dilutes a solution containing one mole of lithium perchlorate in 1.3 moles of ether⁹⁰ by a further 14.2 moles of ether, a volume contraction of 17 cc. per mole LiClO_4 takes place and the molar refraction decreases by 0.91 cc. In this case, strong electric forces are acting between the molecules of the perchlorate and ether.

Extensive experimental evidence concerning the molar refraction of inorganic and organic substances leads⁹¹ to the conclusion that deviations from additivity of the Lorentz-Lorenz equation (46) can be considered as an indication and measure of changes in the electronic systems involved. These changes are due to intra- and intermolecular electric interactions between the parts of the system.

It is beyond the scope of this work to discuss the way in which this point of view has been applied to the deviations from additivity of the molar refraction, dispersion, and volume of molecules, crystals, and solutions derived from ions.⁹¹ However, a few indications may show how exact measurements of molar refractions of organic substances can again be made valuable in the clarification of structural problems.

(a) The Lorentz-Lorenz refraction for infinitely long waves⁹² is considered a measure of the electronic polarizability of the molecule, *i. e.*, of the looseness of its electronic system. The following regularities have been found to have general validity: (1) An electronic system is tightened, *i. e.*, its molar refraction is decreased, by adjacent positive charges. (2) An electronic system is loosened, *i. e.*, its molar refraction is increased, by adjacent negative charges. (3) When, within a given electronic system, the nuclear charge is split or the distribution of the positive charges becomes less symmetrical, the electronic system is loosened, *i. e.*, the molar refraction is increased.

(b) These rules become especially fruitful when combined with the consideration⁹³ of the electron configuration in molecules and ions in respect to the principal quantum numbers. In the isoelectronic series $\text{Ne} (1.00) < \text{HF} (1.9) < \text{H}_2\text{O} (3.75) < \text{H}_3\text{N} (5.67) < \text{H}_4\text{C} (6.58)$, the molar refraction increases from 1.0 to 6.58. In all these particles, the eight outer electrons could be considered to form a closed unit, quantized in respect to the embedded atomic cores. The loosening of this electronic shell is due to the gradual splitting of the $8+$ core of neon leading finally to the $\text{C}^{4+} + 4 \text{H}^+$ cores of methane. The increase (by 0.91) of R from H_3N to H_4C is less than could be expected from the gradation of the preceding molecules of the series.

This could be explained by the more symmetrical tetrahedral distribution of the protons in H_4C . Another possibility is suggested by the relations discussed under (f): The valence electrons of methane, instead of assuming the quantum configuration of neon, may be combined with the protons to form four H^- ions which are strongly polarized by C^{4+} .

⁹⁰ K. Fajans and O. Johnson, *Trans. Electrochem. Soc.*, **82**, 273 (1942).

⁹¹ K. Fajans *et al.*, *Refractometric Investigations*, I-LV (1924-1942). See references 37-63. K. Fajans and N. J. Kreidl, *J. Am. Ceram. Soc.*, **31**, 105 (1948).

⁹² Concerning the reciprocal plotting method of P. Wulff for the extrapolation to $\lambda = \infty$ from measurements in the visible, see reference 61 and pages 1149-1150.

⁹³ See K. Fajans, *J. Chem. Phys.*, **10**, 759 (1942). K. Fajans and T. Berlin, *Phys. Rev.*, **63**, 309 (1943).

(c) *Methane shows an abnormally low refraction also in the series of saturated hydrocarbons, the difference between ethane and methane being 4.8 cc. instead of 4.63. This tightness of the electronic shell in methane is obviously responsible for*

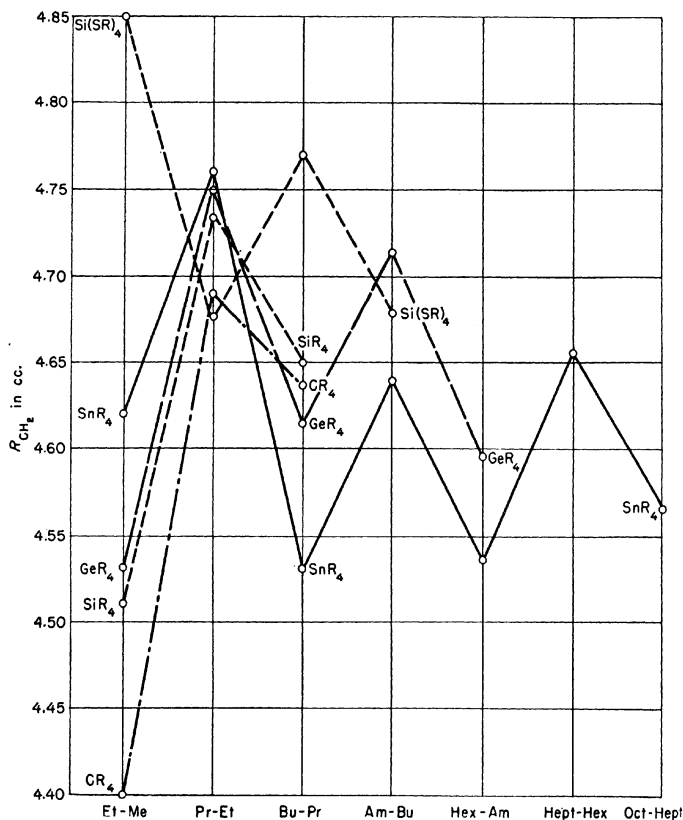


Fig. 2A.—CH₂ increment of the molar refraction (D line) in compounds of Group IV elements.

the relatively high binding energy of this molecule,⁹⁴ and especially for the high energy of dissociation of the first hydrogen atom from it.⁹⁵ On the other hand, for

⁹⁴ F. D. Rossini, *J. Research Natl. Bur. Standards*, **13**, 21 (1934).

⁹⁵ H. G. Andersen, C. B. Kistiakowsky, and E. R. van Artsdalen, *J. Chem. Phys.*, **10**, 305 (1942).

formic acid $R_{\text{exp.}} = 8.56$ is larger than could be expected from R of acetic acid ($12.99 - 4.63 = 8.36$). The looseness of the electronic system of formic acid is reflected also in its binding energy. While the heat of combustion for higher members of homologous series increases by 157 kcal. for each CH_2 group, the difference between acetic and formic acid is 147 kcal. Thus, formic acid has a relatively large heat of combustion, *i. e.*, a relatively small binding energy.

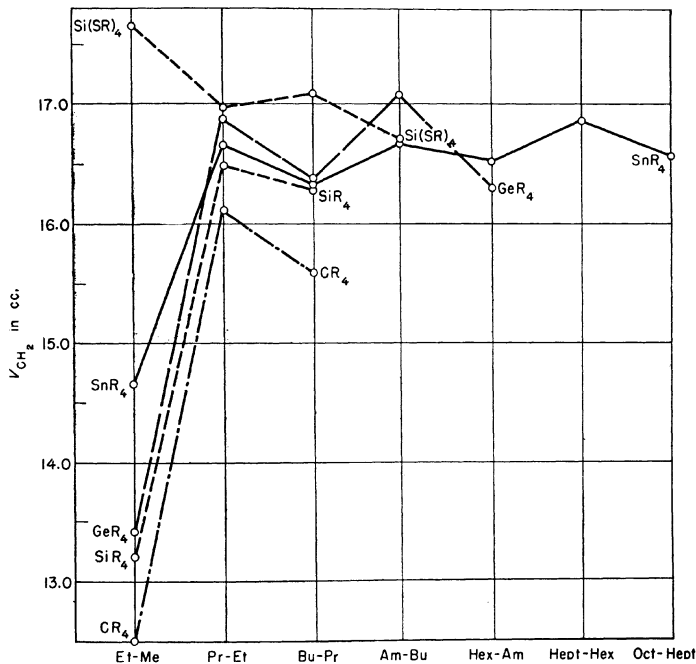
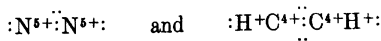
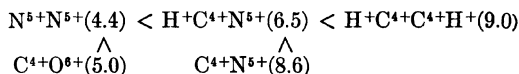


Fig. 2B.— CH_2 increment of the molar volume in compounds of Group IV elements.

(d) The particles, N_2 , CO , CN^- , HCN , and H_2C_2 , have ten valence electrons, which are considered to be subdivided, like those in the neon atom, into a group of two electrons with the principal quantum number I and a group of eight electrons with the principal quantum number II. The electronic structure, for example, of N_2 and H_2C_2 is represented as:



Indicating only the core charges of these five particles, one has the following gradation of the molar refraction:



The gradual increase of refraction between N_2 and H_2C_2 is thus due to the splitting of the first and then of the second $\text{N}^{\delta+}$ core into $\text{C}^{\delta+}$ and H^+ . On the other hand, CO has a higher refraction than N_2 because the distribution of the core charges is less symmetrical in CO; HCN has a smaller refraction than CN^- because the positively charged proton tightens the electronic shell.

(e) The influence of electric charges can be expected to be especially pronounced in organometallic compounds. An examination, undertaken with B. Cook, of the available refractometric data on alkyl derivatives of Si and Sn shows that the values of the increments for the CH_2 group alternate in a homologous series.

In the case of tetraalkyl silicon⁹⁶ compounds the addition of a CH_2 group in the γ (3) position to the central Si atom (i. e., going from the ethyl to the propyl compound) causes a distinctly higher increase in refraction than does the addition of a CH_2 group in either the β (2) or δ (4) position. The corresponding increments with their mean deviations, derived from the number of pairs of values indicated in parentheses, are:

Position	R, cc./mole
$\beta\text{-CH}_2$	4.51 \pm 0.005 (8)
$\gamma\text{-CH}_2$	4.73 _s \pm 0.005 (4)
$\delta\text{-CH}_2$	4.66 \pm 0.00 (2)

In the case of the symmetrical tin tetraalkyls⁹⁷ the addition of successive CH_2 groups in positions 2-8 causes an increase in refraction by the following alternating values:

Position	2	3	4	5	6	7	8
RCH_2 , cc.	4.62	4.76	4.53	4.64	4.53 _s	4.65 _s	4.56 _s

New measurements on symmetrical tetraalkyl germanium compounds^{97a} show the same kind of alternation for positions 2 to 6 of the CH_2 group, with extreme values of 4.53 cc. for position 2 and 4.75 cc. for position 3. The investigation has been extended to molecular dispersion and volume, with results which give additional support to the assumption of alternation in the structural characteristics of successive CH_2 groups in the Group IVb tetraalkyls. The CH_2 increments of molar refraction are represented in figure 2A, those of molar volume in figure 2B.

Since the central Si, Ge, or Sn atom can be expected to have a positive charge, the assumption of alternating polarity seems to offer the simplest explanation of these results. This is supported by the consideration of the refraction of alkyl thio-

⁹⁶ R. O. Sauer, *J. Am. Chem. Soc.*, **68**, 954 (1946).

⁹⁷ W. J. Jones, D. P. Evans, T. Gulwell, and D. C. Griffiths, *J. Chem. Soc.*, **1935**, 39. H. Korsching, *Z. Naturforsch.*, **1**, 219 (1946); *Chem. Abstracts*, **41**, 1902 (1947).

^{97a} Beth Cook, *thesis*, University of Michigan, 1948. K. Fajans, *Chem. Eng. News*, **27**, 900 (1949).

orthosilicates^{97b} in which the first CH₂ group is separated from Si by an S atom. The latter can be expected to have a negative charge, which explains why the alternation of the values due to consecutive CH₂ groups is reversed as compared with the silicon alkyls. One example will illustrate these relations. In the following schematic formulas the + and - indicate only the sign of the excess charge ascribed to the Si and S atoms or to the CH₃ and CH₂ groups involved and not the amount of the charge. The quantum configurations in these compounds are probably Si⁴⁺, S²⁻, (CH₃)⁺ or (CH₃)⁻, and (CH₂)²⁺ or (CH₂)²⁻.

Formula	Difference	R _{CH₃}
SILICON ALKYLs		
$R_3\overset{+}{Si}\overset{-}{CH_3}$	$\overset{-}{CH_2}\overset{+}{CH_3}$ minus $\overset{-}{CH_3}$	4.51
$R_3\overset{+}{Si}\overset{-}{CH_2}\overset{+}{CH_3}$	$\overset{+}{CH_2}\overset{-}{CH_3}$ minus $\overset{+}{CH_3}$	4.73 _s
$R_3\overset{+}{Si}\overset{-}{CH_2}\overset{+}{CH_2}\overset{-}{CH_3}$		
ALKYL THIOORTHOSILICATES		
$\overset{+}{Si}(\overset{-}{S}\overset{+}{CH_3})_4$	$\overset{+}{CH_2}\overset{-}{CH_3}$ minus $\overset{+}{CH_3}$	4.85
$\overset{+}{Si}(\overset{-}{S}\overset{+}{CH_2}\overset{-}{CH_3})_4$	$\overset{-}{CH_2}\overset{+}{CH_3}$ minus $\overset{-}{CH_3}$	4.66
$\overset{+}{Si}(\overset{-}{S}\overset{+}{CH_2}\overset{-}{CH_2}\overset{+}{CH_3})_4$		

The above assumption of alternating polarity of groups α , β , and γ to a central silicon atom can be of help in the interpretation of the reactivity⁹⁸ of chlorine derivatives of silicon alkyls. A Cl atom attached to an α or γ C atom is much less easily hydrolyzed than Cl on a β C atom. This difference in behavior can be related to the expectation that the excess charge of the latter Cl atom should be negative while that of the former should be positive.

(f) In view of the relations discussed under (e) it is of interest to evaluate the best available refractometric data on carbon tetraalkyls.⁹⁹ The designations are

^{97b} H. J. Backer and F. Stienstra, *Rec. trav. chim.*, **51**, 1197 (1932); **52**, 912, 1033 (1933); **54**, 607 (1935).

⁹⁸ F. C. Whitmore *et al.* See, e. g., *J. Am. Chem. Soc.*, **68**, 488, 1881 (1946).

⁹⁹ *Selected Values of Properties of Hydrocarbons*. Am. Petroleum Inst. Research Project 44, Nat. Bur. Standards, June 30, 1945.

analogous to those used for silicon compounds. The fact that the increment for the β position is, as in the silicon alkyls, smaller than that for the γ position indicates that the central C atom in the tetraalkyls also has an excess positive charge and that one can assign to the other groups the following charges: $\overset{+}{\text{C}}(\text{CH}_3)_4$ and $\overset{+}{\text{C}}(\text{CH}_2\text{CH}_3)_4$.

Position	R_{CH_2}	
$\alpha\text{-CH}_2$	4.7	(1) ¹⁰⁰
$\beta\text{-CH}_2$	4.40 \pm 0.01	(3)
$\gamma\text{-CH}_2$	4.68 ₀ \pm 0.004	(2)
$\delta\text{-CH}_2$	4.63 ₀	(1)

It would be premature to draw definite conclusions concerning the polarity of the δ CH₂ in silicon tetraalkyls or the γ and δ CH₂ in the carbon tetraalkyls because the difference between the corresponding R_{CH_2} values and the normal average value 4.63 in a long chain (see page 1170) is not certain enough.

(g) Other series in which molar refraction increments for successive methylene groups in normal alkyl chains, R, have been shown to alternate^{97a} include phenyl alkyl ethers,^{100a} C₆H₅OR, alkyl substituted acetoacetic esters,^{100b} CH₃COCHRCOOC₂H₅, and cyclohexylalkyl alkyl malonates,^{100c} C₆H₁₁(CH₂)₂CR(COOC₂H₅)₂. The sense of the alternation is that expected in a chain attached to negative oxygen in the ethers, and indicates that the chains in the other two series are attached to negative carbon.

(h) Since the *change in distribution of positive charges within an electronic shell* of the definite 10-electron configuration can cause such pronounced differences in the tightness of the shell, it is not surprising that even *chain isomers* show distinct differences in molar refraction.^{78-80, 101} They differ in respect to the spatial distribution of the cores and perhaps also to the quantum configuration of the electronic systems. A rule (not yet published elsewhere) which applies to the comparison of many series in which a normal or iso (isopropyl at end of chain) alkyl chain is attached to oxygen can be stated here as follows:¹⁰¹

The iso compound has a larger intramolecular binding energy (smaller heat of combustion), weaker intermolecular forces (smaller heat of vaporization), smaller density, and smaller refractive index, but a larger molar refraction and molar dis-

¹⁰⁰ Based on the molar refractions 25.4 for tetramethylmethane, F. C. Whitmore and G. H. Fleming, *J. Am. Chem. Soc.*, **55**, 3803 (1933) and 6.60 for CH₄, H. E. Watson and K. L. Ramaswamy, *Proc. Roy. Soc.*, **A156**, 144 (1936).

^{100a} A. I. Vogel, *J. Chem. Soc.*, **1948**, 616.

^{100b} P. Ceuterick, *Bull. soc. chim. Belg.*, **45**, 545 (1936); *Chem. Abstracts*, **31**, 919 (1937).

^{100c} R. Adams, W. M. Stanley, S. G. Ford, and W. R. Peterson, *J. Am. Chem. Soc.*, **49**, 2934 (1927); R. Adams, W. M. Stanley, and H. A. Stearns, *ibid.*, **50**, 1475 (1928).

¹⁰¹ H. S. Mathias, *J. Am. Chem. Soc.*, **72**, 1897(1950).

TABLE VI
PROPERTIES OF NORMAL AND ISOPROPYL ESTERS OF PROPIONIC ACID^a

Compound	Heat of combustion, kcal.	Heat of vaporization, kcal.	<i>d</i>	<i>n</i> _D	<i>R</i> _D	<i>R</i> _γ - <i>R</i> _α
Normal	855.9	8.6	0.8808	1.3936	31.501	0.732
Iso	852.3	8.3	0.8660	1.3872	31.576	0.744
Δ	3.6	0.3	0.0148	0.0064	-0.075	-0.012

^a E. Schjånberg, *Z. physik. Chem.*, **172**, 197 (1935).

persion. As usual, the difference in dispersion is relatively larger than that in refraction. Table VI¹⁰² illustrates the rule.

However, in carbon tetraalkyls in which the alkyl chains are adjacent to positive carbon, the molar refraction of an isopropyl group is smaller by about 0.25 cc. than that of a normal propyl group.^{97a}

There is good reason¹⁰¹ to believe that the nearly generally occurring increase of the Lorentz-Lorenz refraction caused by increase of temperature of a liquid (0.005-0.015% per degree¹⁰³) or accompanying its vaporization (about 1%⁹⁹) is a real indication of the loosening of the electronic shell due to the increase of the distance between the molecules. These facts and the above properties of normal and iso compounds, of compounds belonging to alternating series, and of lithium perchlorate-ether solutions (page 1171) are examples of the parallelism between the change of molar volume and that of molar refraction. The exceptional case of the decrease of the molar refraction and dispersion of liquid water with increasing temperature¹⁰⁴ and of the vapor of acetic acid when the dimer dissociates (page 1168), must be due to specific tightening effects. Displacement of the protons involved in the intermolecular "hydrogen bridges" may be of influence in the case of water.

II. APPARATUS AND MEASUREMENTS

The usual chemical refractometer is a specialized instrument, convenient to use, but not always flexible enough to fit many research problems. Relatively few of the refractometers in the present-day laboratory depart appreciably from the original designs of Abbe (1874) and of Pulfrich (1887), although significant improvements are possible. In order to handle some problems it may be necessary to rely upon instruments which are not ordinarily termed refractometers, such as the spectrometer, the polarimeter, the microscope, and the interferometer. It may even be desirable to modify commercial models, or to construct especially adapted apparatus, using the detailed information available in the literature; this is often well

¹⁰² E. Schjånberg, *Z. physik. Chem.*, **172**, 197 (1935).

¹⁰³ See, for example, K. Fajans, P. Hoelemann, and Z. Shibata (XXI), *Z. physik. Chem.*, **B13**, 360 (1931).

¹⁰⁴ See A. Kruis (LI), *Z. physik. Chem.*, **B34**, 92 (1936).

within the resources of a laboratory equipped with conventional machine tools. While the present article considers the techniques required for best results with commonly available refractometers, it also attempts to point out useful modifications and alternatives.

The accuracy of various methods is of particular concern in the following discussion. It often happens that appreciable errors are made through failure of the observer to control external conditions properly, *e. g.*, in placing the sample, adjusting the light source, etc. Moreover, an individual instrument may fail to meet the optical and mechanical requirements of the measurement. Thus the observed n values of a liquid may show disagreement amounting to several times the expected uncertainty when measurements with different instruments or by different operators are compared (see page 1212). Discrepancies in dispersion measurements are likely to be even more serious (the difference $n_F - n_C$ may be as much as 100% in error if the ordinary Abbe refractometer is used!). Many sources of error can be avoided by recalibration of the instruments with standard substances and by a better understanding of the instruments.

1. Light Sources

The reader is referred to chapter XXI for a systematic treatment of this subject, particularly in regard to sources of continuous radiation in the visible, ultraviolet, and infrared regions as well as to sources of mercury, alkali, and other metal arc radiations. Supplementary information concerning those visible radiations which are especially important in refractometry is given below.

In order to obtain an intense source of radiation containing the desired spectral lines, one relies now chiefly on the use of *electric discharge tubes* of various types. A list and description of the most important lines used in the laboratory or encountered in the literature are given in table VII.

Available commercially¹⁰⁶ are *sodium lamps* which operate directly on 110 volts alternating current. The radiation consists almost entirely of the very intense yellow D_1 and D_2 doublet; the other sodium lines have a negligible intensity, and any neon lines may be filtered out (see pages 1421 and 1528).

The *mercury lamp*¹⁰⁶ which furnishes four very intense lines between yellow and violet is recommended for dispersion measurements. Unfortunately, mercury gives no intense lines at the red end of the spectrum. A mercury-cadmium lamp (G. E. H-4 Type) gives a strong 6438 Å. line.

¹⁰⁶ Sodium Lab-Arc, *General Electric Co.*, Schenectady, N. Y. Gates Sodium Laboratory Arc, *The Emil Greiner Co.*, New York, N. Y.

¹⁰⁶ For example, the General Electric H-4 capillary mercury arc, General Electric Co., or the Hanovia mercury arc, *Hanovia Chemical & Manufacturing Co.*, Newark, N. J.

Various "Geissler" tubes, filled with hydrogen or the noble gases, are also available. These must be operated at a high potential (500 to 15,000 volts).

The usual *hydrogen* discharge tube, widely used in the past because of the simplicity of the spectrum and the availability of the gas, suffers from

TABLE VII
SPECTRAL LINES^a USED IN REFRACTOMETRY

Element	Symbol	Color	λ , Å, in air, 15° C, 1 atm.	$\lambda^2 \times 10^{-10}$ cm. ²	$\nu^2 \times 10^{-23}$ sec. ⁻²
Helium	He _{r1}	Red	7065.19	49.917	17.993
Lithium	Li _r	Red	6707.84 ₆	44.995	19.961
Helium	He _{r2} or r	Red	6678.14 ₉	44.598	20.139
Hydrogen	H α or C	Red	6562.8	43.070	20.853
Cadmium	Cd _r	Red	6438.469 ₆	41.454	21.666
Sodium	D ₁)	Yellow	5890)	34.73	25.86
	D ₂) D	Yellow	5896) 5893		
Helium	D ₃	Yellow	5875.61 ₄	34.523	26.016
Mercury	Hg _{y1}	Yellow	5790.66	33.532	26.785
Mercury	Hg _{y2}	Yellow	5769.60	33.288	26.982
Mercury	Hg _g or e	Green	5460.74	29.820	30.119
Thallium	Tl _g	Green	5350.48	28.628	31.373
Cadmium	Cd _g	Green	5085.82 ₄	25.866	34.724
Helium	He _g or ν	Green	5015.67 ₅	25.157	35.702
Helium	He _b δ	Blue-green	4921.93	24.225	37.075
Hydrogen	H β or F	Blue	4861.33	23.633	38.005
Cadmium	Cd _{b1}	Blue	4799.91	23.039	38.984
Helium	He _b or c	Blue	4713.14 ₂	22.214	40.433
Cadmium	Cd _{b2}	Blue	4678.15	21.885	41.040
Helium	He _v or	Blue-violet	4471.47 ₇	19.994	44.922
Mercury	Hg _{b,v} or g	Blue-violet	4358.34 ₃	18.995	47.283
Hydrogen	H γ or G'	Violet	4340.46	18.840	47.672
Mercury	Hg _v	Violet	4046.56	16.375	54.850

^a Values of λ taken from Landolt-Börnstein, *Physikalisch-chemische Tabellen*, 5th ed., II, p. 816, and Erg. I, pp. 335-366.

several serious disadvantages. Some of the hydrogen lines fade out each time the tube is operated more than a few minutes, while none of the lines are as intense as those from the best sodium or mercury lamps. In addition, slight contamination often leads to the appearance of spurious lines. The tube has a short life, which can be extended only by rather elaborate re-filling procedures; a refillable hydrogen tube is available commercially.¹⁰⁷ Ward and Fulweiler¹⁰⁸ describe a hydrogen tube which can be continuously refilled; it also simultaneously emits lines of the mercury spectrum. A relatively stable tube has been described by Goyan¹⁰⁹ which emits simultaneously the C and F hydrogen lines and the sodium D line, and which can be made in the laboratory. A clean discharge tube filled with gaseous

¹⁰⁷ See the pamphlet, "Reference Manual to Precision Refractometer," Bausch & Lomb Optical Co., Rochester, N. Y.

¹⁰⁸ A. L. Ward and W. H. Fulweiler, *Ind. Eng. Chem., Anal. Ed.*, **6**, 396 (1934).

¹⁰⁹ F. M. Goyan, *Ind. Eng. Chem., Anal. Ed.*, **14**, 60 (1942).

water at the vapor pressure of ice in the range -25° to -30° C. has been found to give an especially intense and stable source of the visible hydrogen lines.¹¹⁰ The advantage lies in the ease with which a high degree of gas purity is obtained. Perhaps the most satisfactory improvements are to be found in the Shell Hydrogen Lamp, which employs a palladium membrane for purification of hydrogen; a description of this lamp has yet to be published.¹¹⁰ Water cooling helps to extend the life of the tube.

The *helium tube*, one form of which is shown in figure 3, is a most convenient, stable source of intense, well-separated spectral lines distributed over the entire visible range. It is especially recommended for use with the Pulfrich refractometer, where the instrument itself serves to separate the spectral lines. This source has the great advantage that the yellow helium

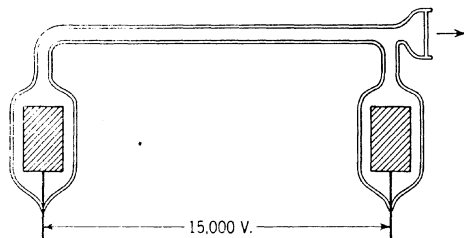


Fig. 3.—Helium discharge tube.

D_2 line has practically the same wave length (5876 Å.) as the commonly used sodium D line. For most colorless organic compounds, the value of n_D , is larger than n_D by only one or two units in the fourth decimal. For maximum intensity, the tube is filled with helium at a pressure of 15 to 20 mm. and is caused to discharge between the hollow, cylindrical nickel electrodes by applying a potential of up to 15,000 volts from an ordinary "luminous sign" transformer (30 ma. secondary). A similar discharge tube, containing only a few millimeters pressure of helium and a small amount of cadmium metal, serves as a source of helium radiation when operated at a few thousand volts, and as a source of numerous intense cadmium lines at higher potentials.

To separate the lines of discontinuous light sources or to isolate certain regions of continuous light, *filters*¹¹¹ or *monochromators*, based on the

¹¹⁰ V. A. Campanile, V. L. Lantz, and L. Lykken, Shell Development Co., *private communication*.

¹¹¹ See "Glass Color Filters," *Corning Glass Works*, Corning, N. Y., "Wratten Filters," *Eastman Kodak Co.*, Rochester, N. Y., and "Jena Colored Optical Filter Glasses," *Fish-Schurman Corp.*, New York, N. Y.

principle of the spectroscope, are used. Sets of filters for the separation of the yellow, green, and blue-violet mercury lines are listed in table VIII. The W77A filter transmits an especially pure radiation ($\lambda = 5460.7$) useful in precision interferometry. For other possible combinations, including liquid filters, see pp. 1421, 1528 and Strong¹¹² and Gibb.¹¹³ A promising development is the interference-type filter.¹¹⁴ It must be kept in mind that filters usually do not sharply separate spectral lines which are closer to each other than about 100 Å. The relative intensities of the various wave lengths transmitted by a given filter can be calculated from its absorption curve¹¹¹ (per cent absorption *vs.* λ) and from the relative intensities¹¹⁵ of the spectral lines in the source.

For the sake of convenience, many refractometric techniques have been adapted to the use of ordinary white light, *e. g.*, that emitted by a tungsten

TABLE VIII
FILTERS FOR MERCURY LINES^a

Wave length, λ ...	4358	5460.7	5769 to 5790
Filter.....	C. (038 + 511) or W. 50	C. (350 + 430 + 512) or W. 62 or W. 77A	C. (349.2 \times std. + 401, ^b $\frac{1}{2} \times$ std.) or W. 22

^a C. refers to Corning glass filters, W. to Eastman Wratten filters.

^b See W. M. D. Bryant, *J. Am. Chem. Soc.*, **65**, 96 (1943); *ibid.*, 128.

lamp, the intensity distribution of which corresponds to a mean wave length ($\bar{\lambda}$) at about 5600 Å. This results in a loss of accuracy, especially where the dispersion of the measured substance is great. By using appropriate filters before a tungsten source, one can obtain colors of sufficient purity for measurements of n_D to an accuracy of a few units in the fourth decimal. Filters which transmit an intense light comparable to the D line of sodium are the W. 64, the W. 73 and the C. (428 + 348).

2. Refractometers Measuring Critical Angle

The most widely used refractometers depend on the so-called critical angle phenomenon, described below.

¹¹² J. Strong, *Procedures in Experimental Physics*. Prentice-Hall, New York, 1942, pp. 372-375.

¹¹³ T. R. P. Gibb, *Optical Methods of Chemical Analysis*. McGraw-Hill, New York, 1942.

¹¹⁴ For example, *Farrand Filters*, Farrand Optical Co., New York, N. Y.; also manufactured by Baird Associates, Cambridge, Mass.

¹¹⁵ G. R. Harrison, *Wavelength Tables*. Wiley, New York, 1939.

Consider a sample m placed on the surface of a prism, P (Fig. 4), having a greater refractive index than m . The sample has the refractive index $n = n_P \sin r / \sin i$, according to equations (1) and (2). If some of the rays at the interface are incident at $i = 90^\circ$ (grazing incidence), the corresponding refracted rays, along r_c , can be precisely located because no rays are refracted into P at an angle greater than r_c , as illustrated in figure 4. In figure 4, the ray traveling along the direction of maximum refraction forms a sharp dividing line (critical boundary) between a light and a dark region

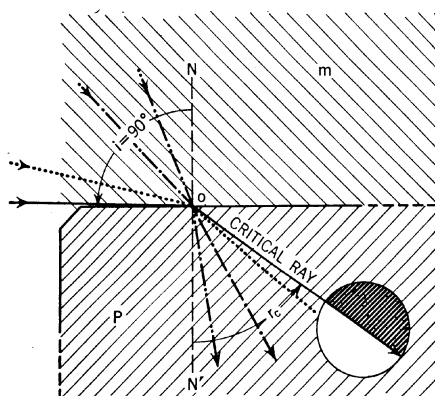


Fig. 4.—Critical boundary inside a prism.

inside the prism. Angle r_c is sometimes called the *critical angle of reflection*, because a ray traveling in the reverse direction of the arrow in figure 4 and striking point o at an angle slightly greater than r_c , would not pass into m but would be totally reflected as though the interface m - P were a mirror.

In practice, the conditions represented by figure 4 are somewhat modified. The value of r_c is not obtained directly. In order to observe the "critical boundary" with a telescope, the rays must pass from the prism into air, suffering, in general, an additional change in direction. Thus, as shown in figure 5a, the observed apparent angle of refraction, r'_c , is smaller than r_c :

$$\sin(90^\circ - r'_c) = \frac{n_P}{n_{\text{air}}} \sin(90^\circ - r_c) = n_P \sin(90^\circ - r_c) \quad (47)$$

Moreover, in actual refractometers, the whole surface of the prism instead of one point may be illuminated with light at or near the grazing incidence.

The telescope, if it consists of perfectly corrected lenses, collects all rays traveling parallel to each other, *i. e.*, all rays with the same r , into one line in the focal plane. Thus, the *critical boundary* shows up in the telescope of most instruments as a sharp, nearly straight line of demarcation between a dark field and a brightly illuminated field having the color of the monochromatic light used. Slightly modified considerations apply to the cases in which white light or sources containing several wave lengths are used (see pages 1184 and 1205).

Table IX summarizes accuracy and range of some commercial critical-angle refractometers.

TABLE IX
MAXIMUM ACCURACY AND RANGE OF CRITICAL ANGLE REFRACTOMETERS

Refractometer	Maximum accuracy, n_D	Maximum accuracy, Δn	Maximum accuracy, $(n_F - n_C)$	Range of n
Pulfrich ^{a, b, c}	$\pm 1 \times 10^{-4}$	$\pm 1 \times 10^{-5}$	$\pm 2 \times 10^{-5}$	1.33-1.61 1.47-1.74 1.64-1.86
Abbe ^{a-f}	$\pm 1 \times 10^{-4}$	$\pm 2 \times 10^{-4}$	$\pm 1 \times 10^{-4}$	1.30-1.70 or 1.45-1.84
Precision Abbe ^{d, f}	$\pm 2 \times 10^{-5}$	$\pm 2 \times 10^{-5}$	$\pm 2 \times 10^{-5}$	1.33-1.64 or 1.36-1.50 or 1.40-1.70
Dipping (immersion) ^{b, d}	$\pm (7 \times 10^{-4})$	$\pm 7 \times 10^{-5}$	1.32-1.54 (6 prisms) ^d

^a Hilger.

^b Zeiss.

^c Bellingham and Stanley.

^d Bausch and Lomb.

^e Spencer.

^f Valentine.

A. PULFRICH REFRACTOMETER

This instrument is particularly useful for differential measurements, including dispersion. Although absolute values obtained with the Pulfrich refractometer are not reliable in the 5th decimal place, largely because of mechanical faults,¹¹⁶ a *comparison* at two wave lengths or of two substances can be made to $\pm 1 \times 10^{-5}$. Solids and liquids of index less than 1.84 can be investigated over a wide temperature range; for $t > 100^\circ \text{C.}$, a specially mounted prism must be used. The Pulfrich refractometer is suited to investigations in which liquid samples must be excluded from contact with air, *e. g.*, hygroscopic liquids or volatile solutions. Although n cannot be read directly, but must be deduced from a set of observations, once the apparatus has been set up a value of n can be obtained about every five minutes.

¹¹⁶ J. Guild *Proc. Phys. Soc. London*, 30, 157 (1917-1918).

The critical boundary is particularly sharp in this type of refractometer even with liquid samples, since it is free from shielding of grazing rays and the full area of the refracting prism may be illuminated, in contrast to the Abbe type (see page 1212).

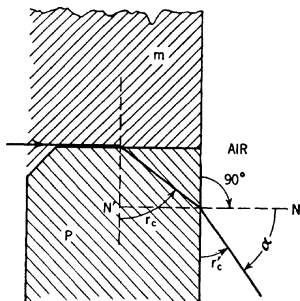


Fig. 5a.—Emergent critical ray.

Optical Principles.—The instrument measures the angle of emergence, α ($\alpha = 90^\circ - r'_c$, Fig. 5a), of the critical ray passing from a 90° prism, P , of index n_P into air. From equations (1) and (2) and figure 5a, $(\sin \alpha)/\sin(90^\circ - r_c) = n_P$ and $n/n_P = \sin r_c/\sin 90^\circ = \sin r_c$; and, by eliminating r_c :

$$n = \sqrt{n_P^2 - \sin^2 \alpha} \quad (48)$$

The "Pulfrich" is not restricted to the use of a monochromatic light source. With the exception of the unusual case that $dn/d\lambda = dn_P/d\lambda$, *i. e.*, that the dispersions of sample and prism are identical,¹¹⁷ there exists a different value of α for each wave length in the source, as illustrated in figure 5b. At first thought, one might expect that in using a multicolored light source the boundary between the dark and light region in the refractometer would be obscured by the superposition of the differently colored noncritical rays which always form a diffuse fringe of color on one side of the corresponding critical ray. However, by confining the incident beam to within very close limits of grazing incidence (compare Figs. 5b and 5c) and choosing sources with spectral lines at well-spaced intervals (Table VII), one obtains a separate sharp critical boundary for each λ . Thus, the Pulfrich refractometer within certain limits serves as its own monochromator. Spectral lines as close as 50 Å. can be dis-

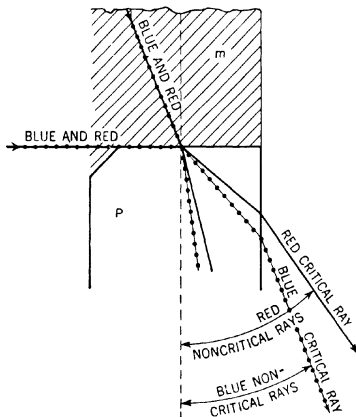


Fig. 5b.—Overlapping of blue critical ray by red rays.

¹¹⁷ W. A. Roth and F. Eisenlohr, *Refractometrisches Hilfsbuch*. Veit, Leipzig, 1911.

tinguished using the ordinary prisms, depending on the dispersions of the sample and the prism; if resolution of the lines cannot be achieved in certain cases, the substitution of a different prism may help. For the behavior of the sodium D doublet, see Guild.¹¹⁶

Construction.—This is shown in figure 6. A pencil of light from S is focused by a condenser, c , to provide rays which just graze the horizontal surface of the beveled right-angle prism, P . An observing telescope, T ,

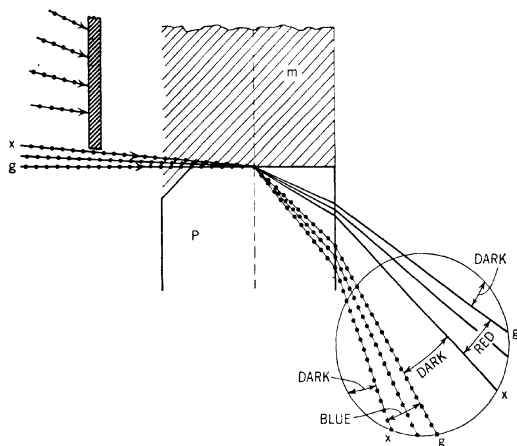


Fig. 5c.—Separation of blue and red critical rays.

with cross hairs H at 45° in eyepiece E , is permanently attached to a large rotatable circle, $G. C.$, graduated in half-degrees of arc (θ_a). The telescope may be inclined at any angle between $\theta = 0^\circ$ and $\theta = 75^\circ$, as indicated by the vernier, when clamping screw, $C. S.$, is loosened, so as to allow location of the critical rays. (At the critical position, the telescope cross hairs are at $\theta = \alpha + \theta_c$; see eq. 52.) For convenience, the refracted rays are first collected by a reflecting prism, P' , in front of the telescope objective, L_3 , causing all rays to be bent at right angles into the telescope so that one can always comfortably make observations from a position facing the plane of the graduated circle. The elliptical stop, st , in front of P' can and should be rotated so as to close off and protect the glass when the instrument is not in use. Another section of the stop can be used to cut off successively light from each half of the prism when making direct comparisons of two substances placed side by side on the prism.

A slow motion of the circle over a limited range ($\approx 5^\circ$ of arc) is provided

by the micrometer tangent screw, *M. S.*, so that the cross hairs can be accurately set at the position of the critical boundary line. The slow motion is transmitted to the circle only when the clamping screw is tightened; the

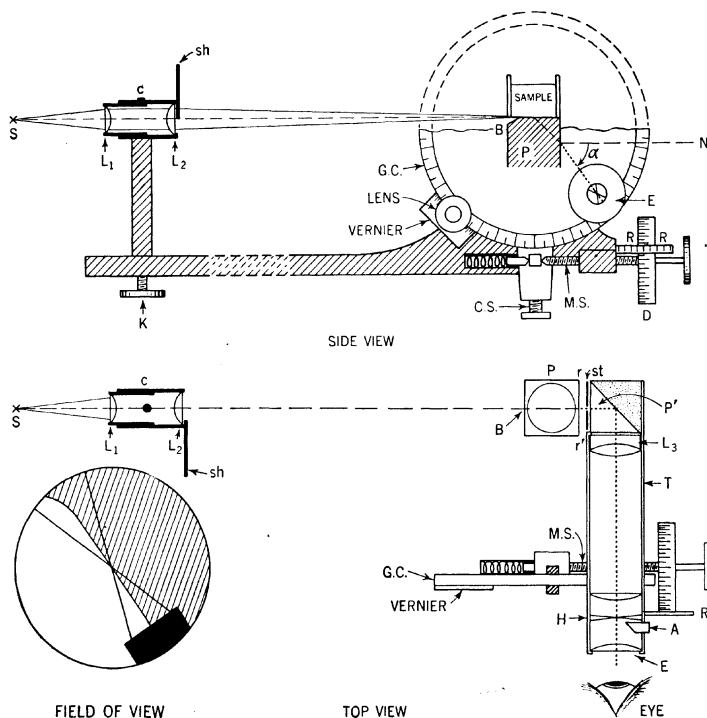


Fig. 6.—Schematic view of the Pulfrich refractometer: α , critical angle of emergence; *A*, Abbe prism (zero point); *B*, prism bevel; *C*, condenser; *C. S.*, clamping screw; *D*, micrometer drum; *E*, eyepiece; *G. C.*, graduated circle; *H*, cross hairs; *K*, condenser screw; *L*₁, *L*₂, condenser lenses; *L*₃, telescope objective; *M. S.*, micrometer screw; *N*, normal line to prism surface; *P*, refracting prism (interchangeable); *P'*, reflecting prism; *r-r'*, refracting edge of prism; *R-R*, reference line carrying revolution counter scale; *S*, light source; *sh*, shutter; *st*, elliptical stop; *T*, telescope.

clamping screw should be kept loose except when the micrometer screw is in use in order to avoid accidental mechanical strain on the telescope mounting. The micrometer screw is also used for the accurate measurement of small angular displacements of the circle, $\Delta\theta$, which is defined by:

$$\Delta\theta = \theta_2 - \theta_1 \quad (49)$$

where θ_1 and θ_2 are the two positions of the *G. C.*

The drum attached to the head of the micrometer screw is marked with 200 equal divisions, the number of divisions going past reference line *R-R* being a measure of $\Delta\theta$ (Fig. 7). One revolution of the drum corresponds to a movement through $20'$ of arc and one drum division to $0.1'$. The drum reading can be estimated to $\pm 0.03'$. For displacements larger than $20'$, the number of revolutions may be counted on a scale along *R-R*. The drum scale, *D*, and the revolution counter scale, *R. C.*, are usually marked in different units. It is advisable to change the units of the *R. C.* scale from degrees to minutes by considering the successive marks on *R. C.* as indicating the 0th, 20th, 40th. . . , division and so on. In this way one

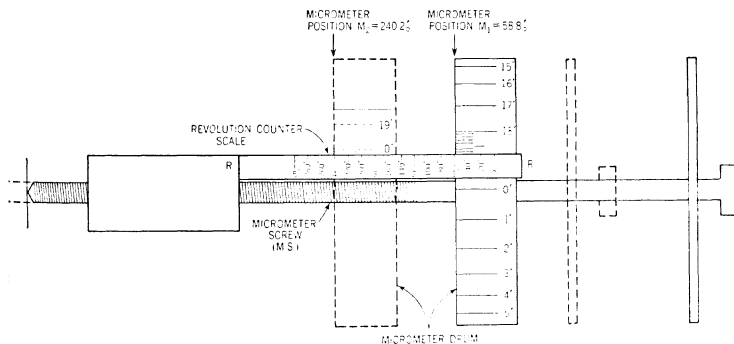


Fig. 7.—Micrometer screw scales of the Pulfrich refractometer labeled to read in minutes of arc.

avoids errors of sign in converting drum positions into values of $\Delta\theta$, for then each *micrometer screw (M. S.)* reading, *M*, is given directly by:

$$M = D + R \quad (50)$$

where *D* and *R* refer to the drum and *R. C.* scale readings in minutes of arc, respectively. We then have simply:

$$\Delta\theta = M_2 - M_1 \quad (51)$$

for any two settings (1) and (2) of the graduated circle, where $M_2 > M_1$. For rapid conversion of scale readings to values of *M*, it is helpful to refer to an enlarged replica of the *R. C.* scale, along which have been placed the values of *R* corresponding to each mark on the scale, as in figure 7.

For large angular displacements, the inaccuracy in the pitch of the

screw and errors in its mounting¹¹⁶ may cause a discrepancy between the true value of $\Delta\theta$, as read from the *G. C.*, and the value read from the *M. S.* scales. This discrepancy may amount to as much as $1.0'$ of arc (1×10^{-4} in Δn), but can usually be ignored if $\Delta\theta < 0.5^\circ$. A rough check on the accuracy of the screw is made by bringing successively two arbitrarily selected marks on the *G. C.*, *e. g.*, 50° and 54° , into coincidence with the reference mark (0 on vernier), each time recording the *M. S.* reading, M_n . To make certain that a discrepancy is not caused by an error in the graduated circle, the operation is repeated for a few other *G. C.* positions (*e. g.*, 54° to 58° , 58° to 62° , etc.). A complete calibration^{44, 116} for very careful work involving a large number of similar comparisons along the whole length of the screw is essential for accurate dispersion measurements. Another, preferable, calibration method is the measurement of $\Delta\theta$ at close intervals along the screw when two standard substances are placed side by side or successively on the prism. The observed deviations of $\Delta\theta$ from the (known) correct value are plotted against the corresponding mean micrometer screw reading, \bar{M} . From this curve, if a sufficiently small $\Delta\theta$ is used, the total correction required for any M can be calculated and plotted. To safeguard against eccentricity of the graduated circle, it is advisable to repeat the calibration at intervals of a few degrees over the whole range of θ likely to be encountered.

The instrument is set up against a dark background in a space in which the temperature stays reasonably constant, or in a thermostated box. An appropriate prism, P , is mounted firmly on the triangular base by tightening the set screw in its mount.

Solid samples are placed on the horizontal prism surface according to the methods described on pages 1199–1200. Various kinds of “refractometer vessels” may be attached to the prism for holding liquid samples (see pages 1197–1199).

Illumination.—With the sample placed on the horizontal surface of P , the light source is fixed a few centimeters from condenser C (Fig. 6) and lens L_1 is shifted back and forth until a sharp (inverted) image of the source is observed on a piece of tissue paper just in front of the prism at B . To obtain maximum intensity, the whole surface of P should be illuminated with slightly converging rays. Consequently, the source image should be at least as wide as the prism, and should be approximately bisected by its horizontal surface. For colored liquids, the image should be raised somewhat higher.¹¹⁷ These conditions will usually not be attained by the single focusing operation described above; to obtain the proper size of the image the relative position of source and condenser will have to be adjusted, followed by a refocusing of the image. The image is displaced up or down by moving condenser screw K . For the final adjustment of the con-

denser, K is turned slowly while viewing the critical boundary through the telescope until maximum sharpness is obtained.

For accurate dispersion measurements (5th decimal), an adjustment of the prism block relative to the telescope may be necessary, since the central ray of the illuminating beam should pass through the center of the telescope objective, L_3 . Proper illumination of the objective is achieved when its aperture is fully or at least symmetrically filled with light.¹¹⁶ To this end the prism block is adjusted on its supporting pillar while examining the "exit pupil" of the telescope with a magnifying lens. The "exit pupil" (or eyepoint) of a telescope is an area, through which pass all rays of the image. It is somewhat smaller than the eyepiece lens surface, and located near the position where the eye would normally be placed to view the image. If a piece of paper is placed at the exit pupil, a bright disk of light is seen. This has an elliptical shape at the exit pupil of the Pulfrich refractometer; it is usually smaller than the superimposed dark image of the elliptical stop (st , Fig. 6). Adjustments should be made until the bright and dark ellipses appear symmetrical with respect to each other.

Temperature Control.—For absolute measurement of n_D of liquids to ± 0.0001 under conditions in which the room temperature is fairly constant, the instrument may be used without special thermal control if the temperature of the sample is measured directly to $\pm 0.2^\circ$ at the time of measurement. In this case, one should know or roughly measure the value of dn/dt (p. 1144) so as to allow interpolation of n at some standard temperature. However, it is usually just as convenient to provide temperature control of the sample to $\pm 0.2^\circ$ by circulating tap water around the prism.

To achieve the highest accuracy ($\pm 1 \times 10^{-5}$) with which it is possible to measure Δn (e. g., $n_F - n_C$, or $n_{\text{soln.}} - n_{\text{solv.}}$), it is necessary to keep the temperature fluctuation of liquid samples within about 0.02° . This requirement is partly taken care of on the standard instruments by the circulation of thermostated water through the prism jacket and through a hollow silver cylinder (fitted with a 0.1° thermometer) which is lowered (rack and pinion) into the open refractometer vessel until it touches the sample. For a modified all-glass "refractometer vessel" providing for temperature control of a completely enclosed liquid, see p. 1198. This device eliminates cooling by evaporation and prevents contamination. It does not provide for the immersion of a thermometer directly into the sample. The temperature may be found accurately enough by taking the average of the readings of thermometers placed in the path of the circulating liquid at inlet i and outlet o of the jacket.

If the working temperature differs considerably ($> 5^\circ \text{ C.}$) from that of the room, disturbing temperature gradients are set up in the prism because

its large vertical surface is necessarily exposed. The most satisfactory way to eliminate such disturbances is to place the entire instrument in an insulated box which can be thermostated to $\pm 1.0^\circ \text{C.}$; this also serves to define the temperature of the reference medium, air. For modifications of the instrument which improve its use at temperatures around 100°C. , see Hoelemann and Kohner⁵¹ and Pesce and Hoelemann.⁵⁸

Conditions for a Sharp Boundary.—When the telescope is in the proper position, the field of view should have the appearance of figure 6 (upper field dark, entire lower field bright, when shutter *sh* is not interposed) if the liquid sample (see page 1200 for solids) is correctly illuminated (see page 1188) with monochromatic light. Contrast may be improved by adjusting the shutter. When the source is a discharge tube furnishing several spectral lines, some or all of these wave lengths should appear as bright colored bands, sharp on the upper edge. The normal order of the bands is violet, blue, green, yellow, and red, looking into the telescope field from left to right (*i. e.*, $\alpha_{\text{red}} < \alpha_{\text{violet}}$). When the dispersion of the sample is near that of the prism (rare case), the order may be inverted or rearranged (see example, page 1197). The width and sharpness of these bands can be changed by shutter *sh* (Fig. 6). When the serrated edge of the shutter is moved so as to cut off more and more of the top part of the light pencil, the colored bands become narrower on the diffuse side, and the whole field of view becomes less intense, while the contrast at the critical boundary is improved. There is never any doubt as to which edge of the band is the critical boundary because its position is never changed by moving the shutter.

Any of the following may cause blurring of the critical boundary: (1) Improper focusing of light source (see page 1188). (2) Poor temperature control, or instrument not at temperature equilibrium. (3) Dirt on refractometer vessel or prism surface, turbidity in the sample. If the incident light is scattered, the intensity of the bands is decreased, and the contrast at the critical boundary is diminished by stray light. Such obstructions may also prevent rays at grazing incidence from entering the prism. This shielding effect can cause appreciable errors in the refractive index. (4) Intensity of light source too low. The critical boundaries for some spectral lines are inherently of lower intensity; for instance, because of this factor, one can obtain n_{He_α} with only about one-third the accuracy possible for $n_{\text{He}_{\beta_1}}$. (5) Overlapping of bands corresponding to neighboring wave lengths in the source. Adjustment of the shutter will eliminate the blurring.

Measurement of Critical Angle of Emergence, α .—The angle which is read from the graduated circle, *G. C.*, when the cross hairs of the telescope are set to coincide exactly with the critical boundary is defined as the ap-

parent critical angle of emergence, θ . The true critical angle of emergence is:

$$\alpha = \theta - \theta_z \quad (52)$$

the *zero point correction*, θ_z , being the *G. C.* reading when the telescope axis (dotted plane, Fig. 6, top view) coincides with a line (*N*, Fig. 6, side view) perpendicular to the vertical prism surface. The deviation from zero, θ_z , of the *G. C.* reading when the telescope is truly at 90° with respect to the prism may be as much as 0.5° .

The true zero position, that is, the true horizontal position of the telescope (*G. C.* at θ_z), is found by locating, with the cross hairs, the light rays which leave the prism exactly perpendicular to its surface. To produce

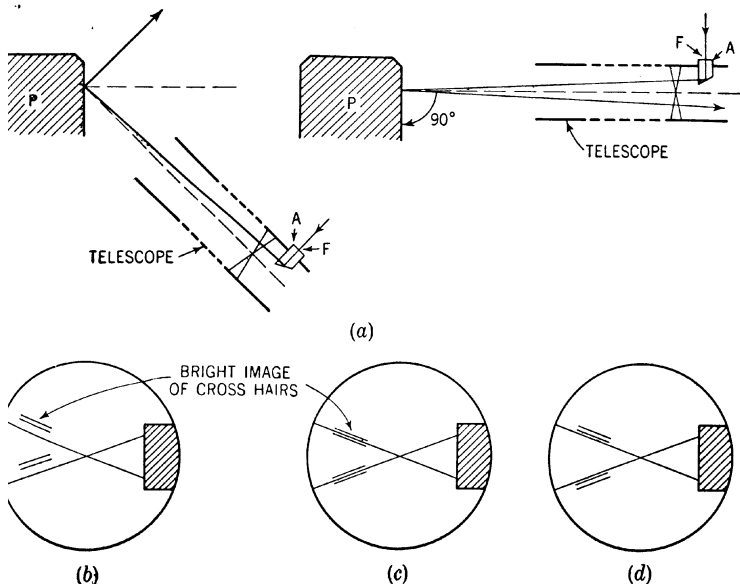


Fig. 8. (a) Alignment of telescope in the true horizontal (90°) position for zero point. The part of the telescope axis passing through the cross-hair intersection in (a) actually makes a 90° angle with respect to the plane of the paper (see Fig. 6). (b-d) Field of view at or near zero point (Pulfrich refractometer).

such rays, the telescope is placed approximately at the horizontal position ($\theta \approx 0^\circ$) and the small Abbe autocollimating prism A (Fig. 6) is illuminated. Light entering the Abbe prism face, *F* (Fig. 8a), is directed to the

vertical surface of prism P , where part of it is reflected back into the telescope, provided its axis is nearly horizontal. In this case, on looking through the eyepiece, a bright image of part of the cross hairs will be observed which is slightly displaced from the cross hairs themselves (Fig. 8b). To obtain optimum brightness and uniform appearance of the cross-hair image, one adjusts the direction and intensity of illumination of face F (Fig. 8a). This is most conveniently done by converging on F with an ordinary lens or telescope the light from a small flashlight bulb. The true horizontal position is now obtained by moving the telescope with the micrometer screw until the cross hairs and its image exactly coincide (Fig. 8c). For many instruments, the optical alignment is such that this coincidence can never be obtained. In this case, the correct position of the telescope is one in which the image appears symmetrical with respect to the cross hairs (as, for example, in Fig. 8d). Consecutive settings on this true zero position can be made to agree within $\pm 0.05'$. The value of θ_z is read directly from the vernier to within $0.5'$ or to within $0.25'$ using the relation:

$$\theta_z = M_z - M_0 \quad (53)$$

(compare with Eqs. 50 and 51), where M_z and M_0 are the micrometer readings when $G. C.$ is set at the true zero position and at the apparent zero position, respectively.

θ_z must be checked from time to time. However, when a number of similar measurements are being made, one can usually assume θ_z to remain constant, unless there is a specific reason for suspecting a change, particularly if one prism has been substituted for another or if samples or vessels have been attached to or detached from the prism, which sometimes is done too forcefully. In general, one should measure the zero point whenever the instrument has suffered some undue mechanical strain, or after it has been subjected to large temperature changes. The changes in θ_z are usually irregular, and during normal use amount to several minutes of arc (4th decimal of n).

To measure the apparent critical angle, θ , move the telescope along with the graduated circle until the critical boundary approximately bisects the field of view. Arrest $G. C.$ with clamping screw $C. S.$ and make the intersection of the cross hairs coincide with the sharp edge of the critical boundary (Fig. 6, lower left) by turning $M. S.$ It is best to make the cross hairs approach the boundary from the bright side of the field, so that the intersection is strongly illuminated during all but the very last part of the setting. With the intense spectral lines (e. g., He_{α}), the settings are reproducible to within $\pm 0.05'$ on the micrometer scale, which corresponds to a precision of better than $\pm 1 \times 10^{-6}$ in n . For a measurement of the absolute value of n , an accuracy of α of $\pm 1.0'$ is sufficient, since inherent in-

strumental errors limit the absolute accuracy to $\pm 1 \times 10^{-4}$. The vernier can be read to $\pm 0.5'$. Several settings should be made. It is advisable to check the instrument by a trial measurement of standard water.

Measurement of Differences ($\Delta n = n_2 - n_1$).—In order to determine differences in n , it is necessary to measure the absolute value of only one (*e. g.*, α_1) of the two critical angles (α_1 and α_2). The value of α_1 should be determined by the displacement method described below since the accuracy in α allowed by direct vernier readings ($\pm 1'$) is not always sufficient to give the fullest possible accuracy in Δn . The measurement of the other critical angle, α_2 , is obtained with the highest accuracy relative to the first, α_1 , if the angular displacement, $\Delta\theta = \theta_2 - \theta_1$ (see Eq. 49), is obtained directly from the micrometer screw while *G. C.* is clamped and if the zero point remains constant. When these conditions hold, $\Delta\alpha = \Delta\theta$, where by definition, for $\alpha_2 < \alpha_1$:

$$\Delta\alpha = \alpha_2 - \alpha_1 \quad (54)$$

In case the two angles, θ_1 and θ_2 , differ by more than the range of the micrometer screw (0.5°), each should be evaluated by the following *displacement method*. The method is based on the principle that one can bring two line segments (— —) into the same line (— —) more accurately than one can interpolate on a vernier. The cross hairs are set on the critical boundary (*M.S.* reading is M_c). Then the micrometer screw is used to bring the zero mark of the vernier in exact coincidence with some nearby whole or half degree mark θ_n on the graduated circle (*M.S.* reading is M_n ; see Eq. 50 for definition of *M*). The angular displacement $\Delta\theta_n$ is:

$$\Delta\theta_n = M_c - M_n \quad (55)$$

and the required angle, θ , is:

$$\theta = \theta_n + \Delta\theta_n \quad (56)$$

It is advisable always to record a rough value of θ from the vernier reading in order to avoid a mistake in the sign of $\Delta\theta_n$. This displacement method allows a reproducibility of about $\pm 0.25'$ for θ , corresponding to an error of $\sim 2 \times 10^{-5}$ in n . For the same purpose, Geffcken and Kohner⁴³ use, instead of the vernier, an additional rigidly mounted telescope with two parallel hairs as fiducial lines. Observing the *G.C.* through this telescope and making the separation of the hairs about equal to the width of the marks on the circle, the angular position of *G.C.* can be reproduced to about $\pm 0.03'$. Such a refinement is justified only when the marks on the graduated circle are narrower and more accurate than is the case for the common instruments, as was the case in the work of Geffcken and Kohner, or when the graduated circle has been completely recalibrated with a variety of standard substances.

The accuracy of the difference, Δn , is limited chiefly by that of $\Delta\theta$. Under the best conditions, Δn can be determined within $\pm 1 \times 10^{-5}$. Although the absolute values of n are accurate to only about $\pm 1 \times 10^{-4}$ due to inherent instrumental errors, these errors are practically constant and nearly cancel out in the calculation of Δn if all measurements are made in succession with the same instrument so that the alignments of prism and telescope remain the same.

Measurements of Dispersion ($\Delta n = n_{\lambda_1} - n_{\lambda_2}$).—Using an appropriate light source (e. g., a helium tube) and with the telescope free to move, the colored band system is located. If the critical boundaries for all spectral lines can be viewed simultaneously (dispersion of sample is relatively close to that of prism), *G. C.* is clamped so that, when the cross hairs are set near the middle of the spectrum, the micrometer drum is about at the midpoint of the *R-R* scale.

The values of n_λ for any two sufficiently intense lines can now be directly compared within $\pm 2 \times 10^{-5}$ by setting the cross hairs on the critical boundaries and finding the corresponding readings, M_{λ_1} and M_{λ_2} , (Eq. 50). For a complete dispersion curve, the values of M are recorded for all lines present. In addition, a measurement is made of the absolute value of α for one spectral line (e. g., He_{D_1}). For weak lines, where the accuracy of the setting is diminished, it may be advantageous to seek a greater contrast at the critical boundary by cutting off some of the incident light with the shutter (*sh*, Fig. 6, p. 1186). For those cases in which the spectral lines are spread so far apart that one cannot view the extreme ends of the spectrum without resetting the graduated circle, it is necessary to measure the position of all wave lengths relative to some intense spectral line in the green or yellow region (e. g., He_{D_2}). In this way, the red and violet lines may be indirectly but accurately compared.

Although the *accuracy* of $n_{\lambda_1} - n_{\lambda_2}$ is limited by any uncertainty in the relative values of $n_{\lambda(P)}$ (Eq. 48) and in the calibration of the micrometer screw (p. 1188), the *reproducibility* is determined chiefly by $\Delta\theta$ and may be as good as 1×10^{-5} .

Comparison of Two Substances.—When Δn is sufficiently small, it is possible to have the critical lines (monochromatic light) for both substances present at the same time in the field of view by placing the (solid or liquid) samples side by side on the prism surface (see pp. 1185, 1198). When the bands overlap ($\Delta\theta$ very small, or light source has many wave lengths), the critical line for each sample can be observed separately by closing half of the aperture in front of the telescope objective.

To obtain the *accuracy* of 1×10^{-5} when the Δn value is large enough (~ 0.005) to require a considerable range of the micrometer screw, a calibration of the screw (see page 1188) is essential. The best *reproducibility* is obtained when the same range of the screw is always used.

If the samples are placed successively on the same prism surface, great care must be exercised to avoid strain on telescope and prism. When Δn is large ($\Delta n > 0.025$), the micrometer screw cannot be used to measure $\Delta\theta$ directly. However, by using the displacement method (p. 1193), it is possible to obtain values of Δn as large as 0.05 with an accuracy of about $\pm 3 \times 10^{-5}$, provided the same reference mark, θ_n , is selected for both θ_1 and θ_2 . The accuracy of still larger values of Δn will depend considerably on the accuracy of the graduated circle. The angular distance between marks on the circle may be checked by comparing measured values of Δn with the known values for various pairs of standard substances.

Conversion of Data to Values of n'_λ .—Values of n_λ^{20} at certain standard wave lengths, λ , can simply be interpolated from the tables of α vs. n_λ^{20} supplied with each instrument. Such tables apply only to the particular

kind of prism glass (indicated on the prism mount) and only at a given temperature, usually 20° C. Usually the tables supply values for the C, D, D₃, e, F, and g spectral lines. Note that the "correction value" given for the C line is always negative, *i. e.*, it must be subtracted from the n value given for the D line. The other correction values are positive.

For wave lengths, λ' , not included in the tables, $n_{\lambda'}^{20}$ is obtained from $\alpha_{\lambda'}$ by means of equation (48); this requires a knowledge of the refractive index of the prism at λ' and 20° C., $n_{\lambda'(P)}^{20}$. The direct measurement of $n_{\lambda'(P)}^{20}$ by means of a precision spectrometer is beyond the means of the ordinary laboratory. The reciprocal plotting method (page 1149) can be used to interpolate values of $n_{\lambda'(P)}^{20}$ from values of $n_{\lambda(P)}^{20}$ published by the manufacturers.¹¹⁸ More reliable is the method of measuring the difference $\Delta\alpha$ (see p. 1193) between the emergent critical angles, α_{λ} and $\alpha_{\lambda'}$, for some standard substance (*e. g.*, water) having accurately known values of n at the two wave lengths. The required value for the prism is calculated by using $\alpha_{\lambda'}$ and $n_{\lambda'(\text{H}_2\text{O})}^{20}$ in equation (48). One finds $\alpha_{\lambda'}$ from the relation $\alpha_{\lambda'} = \alpha_{\lambda} + \Delta\alpha$, where α_{λ} is obtained by substituting the known values of $n_{\lambda(P)}^{20}$ and $n_{\lambda(\text{H}_2\text{O})}^{20}$ in equation (48). In this way $n_{\lambda'(P)}^{20}$ can be conveniently determined with an accuracy of $\pm 2 \times 10^{-5}$ relative to $n_{\lambda(P)}^{20}$.

Prism Temperature Correction.—In order to obtain an accuracy of $\pm 1 \times 10^{-4}$ in n when the temperature of the determination is considerably above or below the standard temperature (usually 20° C.), it is necessary to take into account the effect of the change of the prism index with temperature, dn_P/dt . **EXAMPLE:** Suppose that, for *p*-nitrotoluene at 55° C., using a prism for which $n_{D_1}^{20} = 1.62$ one finds the value, $n_{D_1}' = 1.5383_3$ obtained directly from the tables, which are based on n_P^{20} . The value 1.5383₃ is too low because $n_{D_1}^{55}(P)$ is greater than $n_{D_1}^{20}(P)$ (see Eq. 48), that is, $n_{D_1}^{55} = n_{D_1}' + k$, where k is the correction to be applied. The correction is given by:¹¹⁹

$$k = \frac{n_P}{n'} \cdot \frac{dn_P}{dt} (t - 20^\circ) \quad (57)$$

where t is in degrees C. Knowing that $dn_P/dt = 0.28 \times 10^{-5}$ for this prism for the D₃ line, and substituting in equation (57), we have $k = +(1.62/1.54) (0.28) \times 10^{-5} (55 - 20) = +1.0 \times 10^{-4}$. So $n_{D_1}^{55} = 1.5383_3 + 0.00010 = 1.5384_3$, relative to air at 55° C.

If one must use equation (48) to obtain values of $n_{\lambda'}^{20}$ (*i. e.*, for certain wave lengths, λ'), the effect of prism temperature is accounted for by calculating the corrected prism index at the temperature of measurement, n_P' , from the value at 20° C., n_P^{20} , given with the tables. It is apparent that:

¹¹⁸ See reference 62 for values which have been interpolated for certain Zeiss prisms.

¹¹⁹ See L. W. Tilton, *J. Research Natl. Bur. Standards*, **30**, 319-320 (1943).

$$n_{\lambda}'(P) = n_{\lambda}'^{20}(P) + \frac{dn_{\lambda}'(P)}{dt} (t - 20) \quad (58)$$

The manufacturer usually supplies values of the coefficient $dn_{\lambda}(P)/dt$. The corrected values of n_{λ}' obtained by equation (57) or (58) refer to air at $t^{\circ}\text{C}$. and the prevailing pressure, if the coefficient dn_P/dt corresponds to the *relative* index $(n_P')_{at} = n_P'/n_a'$ (n_a' is the absolute index of air at $t^{\circ}\text{C}$. and prevailing pressure), i. e., if $dn_P/dt \approx (dn_P/dt) - n_P(dn_a/dt)$ is used. However, if the coefficient of the *absolute* index, dn_P/dt , is used in equation (57) or (58), the resulting $(n_{\lambda}')_{at}$ values refer to air at room temperature and the prevailing pressure. The latter frequently encountered mode of expression is correct only in case the effective air temperature is 20°C ., as it is when the telescope is surrounded by air at 20°C . and when the temperature gradient of air near the prism-air interface is uniform. Otherwise it is advisable to maintain the air bath at $t^{\circ}\text{C}$. and express n_{λ}' relative to air at $t^{\circ}\text{C}$. (In either case it may be necessary ultimately to refer the values to dry air at S.T.P.; see page 1142.) The value of $(n_{\lambda}')_{at}$ is approximately 1×10^{-5} less than $(n_{\lambda}')_{at}$ for every 7° of excess of t over 20°C . The manufacturers usually supply tables of dn_P/dt (*absolute* values); Tilton¹¹⁹ gives such data, as well as dn_P/dt (*relative* to air) for various wave lengths, temperatures, and kinds of glass.

The temperature coefficient of the prism depends strongly on the wave length as well as on the type of glass. Thus, the temperature correction is especially important for dispersion measurements. The change in dn_P/dt with temperature is only about 0.1% per $^{\circ}\text{C}$. Therefore it is permissible to assume dn_P/dt is constant in equations (57) and (58), even when 5th decimal relative accuracy is required, provided $(t - 20)$ is less than about 50°C . When working at temperatures at which the correction is large, it is advisable to verify the effect of dn_P/dt by making measurements at various temperatures on a standard substance having an accurately known temperature coefficient of dispersion, for example, H_2O .

SAMPLE CALCULATION OF n_{λ}' , AND $\Delta n = n_{\lambda_1} - n_{\lambda_2}$

Substance: molten *p*-nitrotoluene, $\text{CH}_3\text{C}_6\text{H}_4\text{NO}_2$.

Temperature: 55.00°C . (The air is also at 55°C .)

Prism: $n_D^{20} = 1.62$; $dn_P/dt \times 10^5 = 0.23, 0.28, 0.46$ for $\lambda = \text{He}_{\tau_1}, \text{He}_{D_1}, \text{He}_{\epsilon}$.

Spectral lines: $\text{He}_{\tau_1}, \text{He}_{D_1}, \text{He}_{\epsilon}$.

The graduated circle, *G.C.*, remains clamped during the following operations and the zero point remains constant.

Zero point correction, θ_z (see page 1191): When *G.C.* is set at (1) the true horizontal position and (2) the 0.00° mark, the *M.S.* reading, *M* (page 1187), is (1) 185.5_{\pm} and (2) 185.0_{\pm} . $\theta_z = M_1 - M_2 = 185.5_{\pm} - 185.0_{\pm} = 0.5_{\pm}'$.

Refractive index, $n_{D_2}^{55}$: When *G.C.* is set at (1) position of D_1 critical boundary line i. e., at θ_{D_1} and (2) $30^{\circ} 30'$ mark (θ_n), reading *M* is (1) 168.8_{\pm} and (2) 155.0_{\pm} . By inspection, $\theta_{D_1} \approx 30.7^{\circ}$.

$$\Delta\theta_n = M_1 - M_2 \text{ (Eq. 55)} = 168.8_s - 155.0_s = 13.8'_s$$

$$\theta_{D_1} = \theta_n + \Delta\theta_n \text{ (Eq. 56)} = 30^\circ 30' + 13.8'_s = 30^\circ 43.8'_s$$

$$\alpha_{D_1} = \theta_{D_1} - \theta_z \text{ (Eq. 52)} = 30^\circ 43.8'_s - 0.5'_s = 30^\circ 43.3'_s$$

By interpolation in the tables, one finds that α_{D_1} corresponds to $n'_{D_1} = 1.5383_s$, where n'_{D_1} refers to an assumed prism temperature of 20°C . According to the example on page 1195, the corrected value is: $n_{D_1}^{55} = 1.5383_s + 0.0001_0$; $n_{D_1}^{55} = 1.5384_s \pm 0.0001_0$.

Dispersion.—Data for He_r line (designated below as r): When $G.C.$ is set at (1) position of red line r , *i. e.*, at $\theta_r = \alpha_r + \theta_z$, and (2) position of D_3 line, *i. e.*, at $\theta_{D_3} = \alpha_{D_3} + \theta_z$, ($\alpha_{D_3} = 30^\circ 43.3'_s$), reading M is (1) 195.0_s and (2) 168.8_s. By inspection, $\alpha_r \approx 31.2^\circ$.

$$\Delta\theta = \Delta\alpha = M_1 - M_2 \text{ (page 1193 and Eq. 51)} = 195.0_s - 168.8_s = 26.1'_s$$

$$\alpha_r = \alpha_{D_3} + \Delta\alpha \text{ (Eq. 54)} = 30^\circ 43.3'_s + 26.1'_s = 31^\circ 9.5'_s$$

Most tables do not give values of n_r . Then, in order to use equation (48), we must first find $n_r^{55}(P)$ of the prism. If we have determined (see page 1195) that $n_r^{20}(P) = 1.61523$, we can calculate its value at 55°C . from equation (58). We substitute the appropriate value (0.23×10^{-5}) of dn_P/dt and obtain $n_r^{55}(P) = 1.61523 + 0.23(55 - 20) \times 10^{-5}$, or $(n_r^{55}(P))^2 = 2.60922_s$. Equation (48) becomes

$$n_r^{55} = \sqrt{2.60922_s - \sin^2(31^\circ 9.5'_s)} = 1.5301_s,$$

this absolute value being correct within about $\pm 1 \times 10^{-4}$. The difference, $n_{D_3} - n_r = 0.00825$, is correct within about $\pm 3 \times 10^{-5}$ (page 1194).

Dispersion.—Data for He_v line (designated as v): When $G.C.$ is set at (1) position of violet line, *i. e.*, at $\theta_v = \alpha_v + \theta_z$ and (2) position of D_3 line ($\alpha_{D_3} = 30^\circ 43.3'_s$) reading M is (1) 235.8_s and (2) 168.8_s.

$$\Delta\theta = \Delta\alpha = 235.8_s - 168.8_s = 67.0'_s$$

$$\alpha_v = 30^\circ 43.3'_s + 67.0'_s = 31^\circ 50.3'_s$$

Using equation (58) as before, we can find $(n_v^{55}(P))^2 = 2.69157_s$. Accordingly:

$$n_v^{55} = \sqrt{2.69157_s - \sin^2(31^\circ 50.3'_s)} = 1.5534_s$$

The dispersion of *p*-nitrotoluene at 55°C . can then be expressed as $(n_v - n_r)^5 = 0.02328 \pm 0.00005$. It is interesting to note that, because the dispersion of this compound (0.02328) is almost as great as the dispersion of the prism (0.02537), one observes an abnormal sequence of the colored critical boundaries violet, red, and yellow instead of violet, yellow, and red.¹¹⁷

Handling of Liquids.—A layer of liquid about 0.5 mm. thick ($\cong 0.2$ ml.) covering the entire horizontal prism surface is sufficient for the most accurate measurements. If a smaller area of the prism were used for very small samples, there would be a proportionate decrease in the intensity of the critical boundary. Correspondingly, the symmetry of illumination of

the telescope aperture would become more important.¹¹⁶ Ordinarily, about 4 ml. of sample is used, half of this for preliminary rinsing of the glass surfaces until a constant value of n is obtained.

A simple cylindrical tube, ground at the base to fit the prism (standard equipment), serves to confine the liquid and provides a vertical window for the incident light rays. When this arrangement is used, the vessel may be filled by a pipette, but the liquid should be removed with a piece of lens paper to avoid scratching the relatively soft prism surface. Filter paper should not be used. The simple tube arrangement is not always satisfactory.

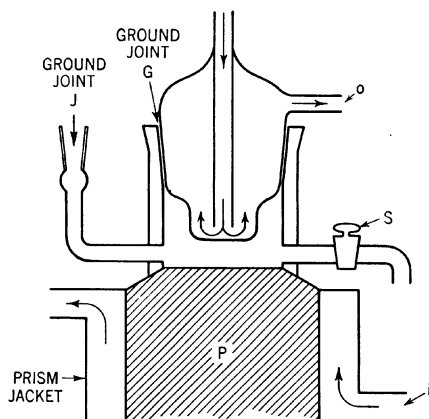


Fig. 9.—Improved vessel for liquids (Pulfrich refractometer). Thermostated water enters at inlet i and leaves at outlet o .

An *improved vessel* by Geffcken and Kohner⁴³ (Fig. 9) allows rapid filling and draining while excluding the atmosphere. A wash-bottle type of flask is connected to the vessel through ground joint J and liquid is forced into the space above the prism by gas pressure. Rinsing is facilitated by providing an air outlet (e. g., through a small channel in ground joint G) which allows the vessel to be filled while stopcock S is closed. After a measurement, pressure can be applied through J to remove most of the liquid, and the interior of the vessel then can be cleaned rapidly by rinsing with pure solvent and drying with a stream of pure air.—A special *double-chamber cell* is useful for the direct comparison of two liquids. The usual tube is provided with a black glass partition perpendicular to both prism faces which bisects the polished horizontal prism surface.—If a suitable cement can be

found, the vessels should be sealed to the prism bevel to prevent loss by evaporation and to increase mechanical strength. It is essential that the double-chamber type be tightly cemented. In selecting a cement, the following considerations are of prime importance: (1) The *dry* cement should not be soluble in the liquid, or should have only a very slow rate of dissolution; and (2) the dry cement must be easily softened by some solvent. The vessel should never be removed by force; otherwise, the zero point will change, and the prism may become seriously chipped. The cement may be tested in the following way. When the cement is dry, a minimum amount of the liquid under investigation is filled into the vessel. The vessel is covered tightly, and the instrument thermostated. An excessive interaction of liquid and cement is revealed by a change in θ over a period of several hours. Occasionally, a cement is attacked by a liquid only during the first few hours of contact, and can be used thereafter.

Handling of Solids.—*Large Pieces of Sample.*—The material must be homogeneous and have two mutually perpendicular faces, one of which must be plane and clear (or capable of being sufficiently polished). The other face, which merely acts as a window for the incident light, can have an irregular nonplanar surface. The two faces should intersect in a sharp line so that the "window" will allow light to enter the crystal at grazing incidence. Samples with appropriate faces can often be obtained by crushing a large specimen which has at least one clear plane surface. The largest suitable piece is selected from the fragments which have sharp edges.

The clear plane face is brought into optical contact with the horizontal refractometer prism face by placing a very small drop of an appropriate liquid on the clean prism surface and carefully pressing the solid into place so that a very thin, bubble-free film separates the two surfaces. The liquid used to provide optical contact must have an index of refraction *greater* than that of the solid to be measured. 1-Bromonaphthalene ($n_D = 1.68$) is commonly used. It does not matter if this liquid slowly dissolves the solid. Excess liquid which happens to collect near the "window" is removed; it causes blurring of the critical boundary and is the source of a serious error if rays at grazing incidence become shielded.

If the liquid film contained between the sample and prism has the shape of a wedge (which it almost invariably does) it may cause an appreciable error¹²⁰ by altering the effective angle of the refractometer prism. An error as great as 2×10^{-4} can arise from this wedge effect. The latter is minimized when the refractive index of the contact liquid does not greatly exceed that of the solid sample. However, the only certain way to eliminate

¹²⁰ For a quantitative discussion of the wedge effect, see: L. W. Tilton, *J. Research Natl. Bur. Standards*, **30**, 323 (1943); *J. Optical Soc. Am.*, **32**, 376 (1942); and J. Guild, *Proc. Phys. Soc. London*, **30**, 157 (1917–1918).

this source of error is properly to control the shape and orientation of the liquid layer.

We use the method of interference fringes to observe the slope of the liquid wedge. Monochromatic light passing through or reflected from the two broad surfaces of the wedge forms a set of interference fringes consisting of equally spaced light and dark bands parallel to the edge of the wedge. These bands can be observed either by directly viewing the broad surface of the wedge from above (assuming the sample is sufficiently transparent) or by inspecting the exit pupil (see page 1189) of the telescope with a simple magnifying lens. The greater the number of bands observed per unit length of wedge, the greater are the wedge angle and the error. By exerting just the right pressure on various parts of the sample, it would be possible to decrease the slope of the wedge until the interference fringes disappear, that is, the liquid layer becomes a plane-parallel window which causes no error. However, in actual practice it is not necessary to eliminate the wedge completely. It has been calculated¹²⁰ that one-third of a fringe of yellow light per centimeter length of wedge (viewed through the exit pupil) corresponds to an error of about 1×10^{-8} in the index of refraction of the sample. When viewed from above the sample, this number is one or two fringes per 1×10^{-8} error in n , depending somewhat on the index of the liquid relative to the prism. Thus, for accurate dispersion measurements it would be necessary to adjust the wedge until not more than a single fringe could be seen in the exit pupil (the prism surface allows the formation of a wedge about 2 cm. long). For measurements of n_D to 4th place accuracy, as many as six to eight fringes can be tolerated.

Practically no error will result if the wedge has its sharp edge *perpendicular* to the refracting edge of the prism. The refracting edge of the prism (rr' in Fig. 6) is perpendicular to the optic axis of the collimator. Hence, even for a fairly steep wedge, it suffices to exert pressure on the sample so that the interference fringes become perpendicular to the refracting edge, *i. e.*, perpendicular to the critical boundary, if viewed at the exit pupil. The wedge should be tested both before and after a measurement to insure against a change.

Concerning fine interference bands parallel to the critical boundary (Herschel fringes), appearing when a plane-parallel layer of liquid is enclosed between the prism and another plane surface, see Tilton.¹²⁰

Powders.—A convenient and accurate method of measuring n (to ± 0.0002) for small amounts of isotropic powders has been suggested by Le Blanc.¹²¹ When particles of such a solid are immersed in a liquid of identical index of refraction it is possible to observe a characteristic sharp

¹²¹ M. Le Blanc, *Z. physik. Chem.*, 10, 433 (1892).

critical boundary and thus to measure n_b ; when the two media have slightly different values of n , the boundary is diffuse, or disappears.

Two liquids (L_I , L_{II}) are selected which do not appreciably (or rapidly) dissolve the solid in question, one (L_I) with a lower and the other (L_{II}) with a higher index than the solid. For many organic compounds, one can use saturated aqueous potassium mercuric iodide ($n_{II} = 1.73$) and H_2O ($n_I = 1.33$). Kaiser and Parrish¹²² discuss immersion liquids for solids. Enough of L_I is filled into the refractometer vessel so that the contents can be stirred. A few milligrams of the powder are added to form a fine deposit on the prism surface (too much sample would scatter all the light). The average cross section of the particles may vary from 1 to 0.01 mm. The stirring is commenced and L_{II} is slowly dropped in while an observation is made through the telescope, with monochromatic light. At first, the entire field is dark for all positions of the telescope. When $n_{liq.}$ comes fairly close to n_{solid} , one can observe a broad diffuse band of light, bordered on

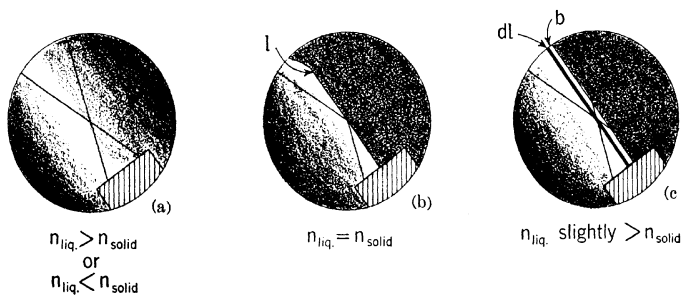


Fig. 10.—Field of view in Le Blanc's powder method for isotropic powders.

either side by a dark region (see Fig. 10a). When $n_{liq.} = n_{solid}$, the band develops one fairly sharp border line, l , toward the upper (right) part of the field of view (Fig. 10b). On a further very small addition of L_{II} , the light and dark areas become separated by an intense bright band, b , and an adjacent fine dark line, dl , as shown in figure 10c. The cross hairs can be most accurately set on line dl , which gives a value of n_{solid} only slightly greater ($\sim 1 \times 10^{-4}$) than the true value. During the final stage of gradually increasing $n_{liq.}$, it may be more convenient to depend on the evaporation of L_I if this is the more volatile component. A slow drift

¹²² E. P. Kaiser and W. Parrish, *Ind. Eng. Chem., Anal. Ed.*, **11**, 560 (1939). See also E. M. Chamot and C. W. Mason, *Handbook of Chemical Microscopy*, 2nd ed., Vol. I, Wiley, New York, pp. 373-375, 381-382; T. R. P. Gibb, *Optical Methods of Chemical Analysis*, McGraw-Hill, New York, 1942, pp. 251-253; and Chapter XV, p. 934.

of several degrees in the liquid temperature is permissible during the procedure as long as the critical boundary remains sharp at the actual time of measurement, since dn/dt for most solids is small.

Modified Pulfrich Refractometer.—The Pulfrich type of instrument has been redesigned by *Bellingham and Stanley Ltd.*, London. Figure 11 shows the external features of this improved, convenient model. The en-

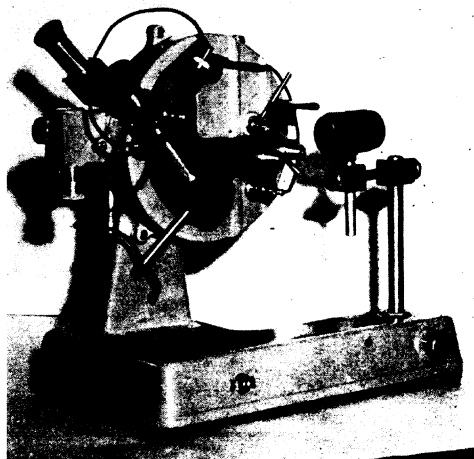


Fig. 11.—Modified Pulfrich refractometer (B. and S. "Critical Angle Refractometer"). Courtesy *Bellingham and Stanley Ltd.*

closed graduated circle is made of glass, which minimizes corrosion of the fine scale marks and allows the use of transmitted light for observations. The vernier has been dispensed with, fractions of a degree being determined by the more accurate micrometer screw method. A precision of one second of arc in the angle θ is possible. To avoid errors caused by eccentricity of the graduated circle, the optical system of the reading microscope is arranged so that opposite sides of the circle are seen together in the field of view. Liquid samples may be placed in a water-jacketed cell designed to maintain a constant temperature and to prevent evaporation. Also available are cell and prism combinations which allow direct comparison of two liquids placed side by side. With this arrangement an accuracy of 1×10^{-5} in Δn can be achieved.

B. ABBE REFRACTOMETER

Like the Pulfrich, the Abbe refractometer measures the critical angle of refraction (or reflection) relative to a glass prism. Its usual type requires a sample of only about 0.05 ml. of liquid and is designed for a maximum speed, convenience, and simplicity compatible with fairly high accuracy (n_D accurate to ± 0.0002). The *scale reads directly in n_D* . The instrument is ordinarily used with white light. With such a source, the dispersion, $n_F - n_C$, can be obtained with an accuracy of from 1 to 30%, depending largely on the compensator¹²³ (see page 1209). The range of the available models¹²⁴ ($n = 1.30$ to 1.71 and 1.45 to 1.84) is adequate for most organic liquids, but the range of a given instrument cannot be changed except in one case (p. 1214). The convenience of the "Abbe" is somewhat offset by certain limitations. It is not well suited to the accurate measurement of solutions with a volatile component (page 1206) or of powders. On the other hand, some models are applicable to opaque solids which cannot be measured with the Pulfrich. The usual instrument is not suited to measurement of n for lines other than the sodium D (see page 1209). However, a recent version, a precision Abbe refractometer (see page 1209), is almost as accurate as the Pulfrich refractometer for dispersion measurements, and surpasses all other commercial instruments in the accuracy for absolute values of n (5th decimal).

Ordinary Instrument.—The ordinary Abbe refractometer has become a standard instrument; models which differ only in small details from the early Zeiss instrument are offered by a number of manufacturers.¹²⁴ Figure 11a illustrates the path of the rays and the mechanical arrangement for locating the critical boundary. The upper, P , of two big prisms is mounted rigidly on a bearing, B , which has an arm, or alidade, A , extending at right angles to the upper face of P . The rotatable arm A carries a small plate, pl , with a reference mark, R , which can be viewed through a magnifying glass, M , mounted above the plate. The telescope is firmly mounted on the arm of a similar bearing, B' , which also rotates about the axis of the prism-bearing B . Telescope arm A' carries a curved scale (sector scale S) which is closely fitted against the alidade plate so that scale S and reference mark R can be viewed simultaneously with the magnifying glass. Thus one can rotate the refracting prism (P) relative to the axis ($T-T'$) of the telescope, while the telescope itself can be moved to any convenient inclination. Some instruments are provided with a rack and pinion mounted on S for moving the prism; in others, it is rotated by hand except in the final stages

¹²³ L. E. Dodd, *J. Optical Soc. Am.*, **22**, 477 (1932).

¹²⁴ *Bausch & Lomb Optical Co.*, Rochester, N. Y.; *Bellingham and Stanley, Ltd.*, London; *Ercon Corp.* (Zeiss), New York, N. Y.; *Spencer Lens Co.*, Buffalo, N. Y.; *Adams Hilger, Ltd.*, London; *Industro Scientific Co.*, Bellmore, Long Island, N. Y.

of adjustment, when a slow-motion tangent screw is used. A rotation of the prism is accompanied by a motion of R along scale S . The reading of the sector scale relative to R is a measure of the angle, r , between the normal line, $N-N'$, to the prism surface and the telescope axis, $T-T'$. When a properly illuminated sample is placed on the lower face S' of prism P and the telescope cross hairs are set on the critical boundary, the resulting angle.

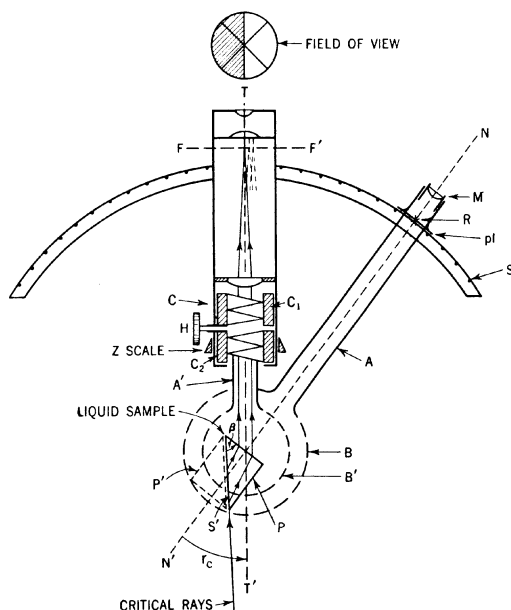


Fig. 11a.—Abbe refractometer: A , alidade; A' , telescope arm; B , prism bearing; B' , telescope bearing; C , compensator; C_1 , C_2 , Amici prisms; $F-F'$, focal plane; H , milled head; M , magnifying glass; $N-N'$, normal line to upper surface of prism; P (upper) refracting prism; P' , auxiliary prism; pl , alidade plate; R , reference mark on pl ; r_c , emergent critical angle; S , sector scale; S' , sample-prism interface; $T-T'$, telescope axis.

r_c is the emergent critical angle characteristic of the sample, of P and of the air. Analysis shows that r_c is related to n of the sample by the equation (59):

$$n = \sin r_c \cos \beta + \sin \beta \sqrt{n_p^2 - \sin^2 r_c} \quad (59)$$

where β is the prism angle and n_p its index of refraction. Actually, the

sector scale is calibrated in terms of n_D^{20} (or even in terms of concentration of aqueous sugar solution, etc.).

Although white light is used, n is obtained for the sodium D line. Ordinarily, with white light, the critical boundary is diffuse and has the appearance of a rainbow because of the divergence of critical rays having different wave lengths (dispersion effect). In this case, the value of n is not accurately defined. The width of the color band depends on the relative dispersion of sample and prism. The critical boundary can be sharpened and its color removed by means of the compensator, C (Fig. 11a), as follows: If two identical Amici prisms, C_1 and C_2 , are properly placed in the path of the critical rays, a dispersion of these rays results which is equal and opposite to that caused by the sample plus prism. Thus, by collecting the divergent critical rays so that all wave lengths are superimposed on one line in focal plane $F-F'$ of the telescope, the Amici prisms compensate for the dispersion of the rays leaving the big prism, and the resultant critical boundary is practically white. The compensator prisms are constructed so that rays for the sodium D line are not deviated, while the dispersion introduced for other wave lengths is proportional to the angle, σ , through which one of the C prisms is rotated relative to the other. The correct amount of compensating dispersion is obtained by rotating the milled head, H , until the critical boundary is sharp and colorless. There are two positions of the compensator, 180° apart, which give an achromatic boundary. If the sodium D line is used for the illumination, the compensator is, of course, unnecessary. Other monochromatic sources are not suitable unless an elaborate calibration is made (see page 1209).

Measurement of Liquids.—PLACING AND ILLUMINATING THE SAMPLE.—An auxiliary prism, P' (Fig. 11a), below the refracting prism serves to illuminate and confine the sample. It is hinged so that its ground-glass hypotenuse is either clamped almost in contact with the refracting prism, or swung out. When a drop of liquid is placed on the ground surface and the two prisms are locked together with the clamp, a thin film (0.1 mm.) of liquid is held against upper prism P . When the ground face of P' is illuminated by tilting the mirror, some of the light scattered at its mat surface passes into the liquid at nearly grazing incidence, and produces a critical boundary. The same light (day or electric) should be used for measurements and calibration.

PRECAUTIONS.—(1) Clean the delicate prism surfaces with alcohol, touching them only with soft lens paper. Leave no lint. Rinse with the liquid under investigation. The liquid itself must be clear or the boundary line becomes blurred. For somewhat opaque liquids, see page 1206. (2) Make certain, by looking through the top surface of P , that the liquid is spread evenly between the prisms when clamped together; bubbles in the

liquid film can also be detected by looking at the exit pupil (p. 1189) with a hand magnifier. For hygroscopic liquids, or those with a volatile component, hold the two prisms barely apart during filling, and lock them as soon as possible. Lack of protection for the sample is often a serious disadvantage of the Abbe. Even after locking of the prisms, the sample may acquire moisture or evaporate. (3) Allow time for establishment of temperature equilibrium. (4) Tilt the mirror to give maximum illumination of the field of view. A change in the position of the mirror or light source does not change the position of the boundary, if it is the true critical boundary. If spurious lines are observed, make the light source more uniform in intensity. (5) Incline the prisms by moving the alidade until the critical boundary is in the middle of the field; focus the eyepiece if necessary; and achromatize the boundary by rotating the compensator. With a slow motion of the alidade, cause the sharp colorless critical boundary to cross exactly the intersection of the cross hairs, as in figure 11a. The reproducibility of the individual readings on the sector scale is ± 0.0001 . Another setting on the critical boundary should be made with the compensator in the other achromatizing position. The two index readings will almost always differ. Tilton¹²⁵ has pointed out that the mean value is free from the particular instrumental error giving rise to this effect. (6) After a measurement, clean the prisms at once, since the prism orientation can change by contact of the mounting cement with various liquids.¹²⁶

Measurement of Solids.—The method of attaching a large solid sample to the lower surface of the Abbe prism is identical with that discussed on p. 1199. It should be recalled that an improperly oriented wedge of the liquid used in making contact between sample and prism may cause errors in the 4th decimal of n . The auxiliary prism, lowered as far as possible, reflects rays into the sample "window" at grazing incidence. Otherwise the procedure is identical with that for liquids except that temperature control need not be as exact.

OPAQUE OR COLORED SOLIDS AND LIQUIDS.—Some models of the Abbe refractometer provide a refracting prism with a window for illuminating the lower surface of P from within. By this means, light can be *reflected* from this surface, S' , into the telescope when the surface is covered with an opaque or a transparent sample. A sharp critical boundary is observed when the telescope axis is at the critical angle of reflection, which is equal to the critical angle of refraction (see page 1182). The intensity of the field is somewhat less than with light entering a transparent sample by refraction at grazing incidence. Otherwise the observations and measurements are the same in both cases.

¹²⁵ L. W. Tilton, *J. Optical Soc. Am.*, **32**, 373 (1942).

¹²⁶ L. W. Tilton, *J. Research Natl. Bur. Standards*, **30**, 311 (1943).

Temperature Control and Corrections.—Both prisms are mounted in a jacket through which thermostated water can be circulated. A thermometer should be placed in the stream which is leaving, rather than entering, the jacket. For most liquids, the temperature must be controlled to $\pm 0.2^\circ$ to realize the full accuracy of the instrument. The thermostating should commence about 20 minutes before using the instrument to insure temperature equilibrium in the prisms. The constancy of the first measurement should be checked by observations over a period of about five minutes. At first, the critical border may appear blurred, due to temperature gradients. Once equilibrium has been established, it takes only a few minutes for each new sample to reach the correct temperature. Grosse¹²⁷ has described a method for using the Abbe refractometer down to -50° C. for volatile substances.

For precise work considerably above or below room temperature, a correction should be applied to the scale readings because of a change in refractive index of the prisms. The scale values on the instrument are almost always given for a temperature of the prism of 20° C. (i. e., $n_p \equiv n_p^{20}$ in Eq. 59). The prism temperature correction is negligible in the range from about 15° to 25° C. for a calibration temperature of 20° . Between 25° and 35° the true index, n' , of the liquid is approximately 1×10^{-4} greater than the apparent value, n' , obtained directly from the sector scale. Similarly, for the range between 15° and 5° , n' is about 1×10^{-4} less than n' . The correction to n' depends somewhat on the absolute value of n , that is, it is different for different parts of the sector scale. The correction to n' depends also on the temperature and temperature gradient of the air at the prism surface¹²⁶ although the n' values are probably independent of air temperature if the telescope is maintained at 20° C.

Because the temperature of the compensating prisms must also be taken into account, it is not convenient or always reliable to calculate a prism temperature correction from the known temperature coefficient, dn_p/dt , of the prism glass. A method of calculation for the Hilger instrument is given by Tilton,¹²⁶ who also points out the limitations of such a procedure. It is preferable to calibrate directly the sector scale at the working temperature (t° C.) by measurements with standard substances (see Table I) whose values of n_b at t° C. (relative to air at the prevailing temperature and pressure) are known, or whose dn/dt values are known in addition to n' ; thermostating the entire instrument with an air bath is then desirable.

Testing the Instrument.—In view of the many construction elements which can lead to errors in individual instruments, and of accidental changes in calibration,^{125, 126} it is advisable to check the compensator and the sector

¹²⁷ A. V. Grosse, *J. Am. Chem. Soc.*, **59**, 2739 (1937).

scales from time to time. A description of simple tests for various common defects of Abbe refractometers is given by Tilton.^{125, 126} It is possible to have tests and recalibrations made by the National Bureau of Standards (see p. 1155). The sector scale readings are tested by measurements with a set of standards (page 1154). Test plates of glass, furnished by the manufacturers, are convenient standards, although it is preferable to use them only when calibrating the instrument for measurements of solids. Test plates may be sent to the *National Bureau of Standards*, Washington, D. C., for calibration. The value of n_0 obtained for a liquid is usually too low if the instrument is adjusted to give the correct value for a solid of the same n . The discrepancy can be as much as 3×10^{-4} for liquids of low n , due to shielding of rays at grazing incidence by the auxiliary prism and/or the presence of "Herschel fringes."^{125, 128}

Any discrepancy between the known n value of the standard and the value n' read from the sector scale can be corrected for in two ways: (1) With the standard on the prism, the alidade is set at the scale reading which represents the correct value of n . The critical boundary is then brought into exact coincidence with the cross-hair intersection by turning with a small screw driver or special key the small screw located in the telescope just above the compensator. This effects a shift in the position of the telescope objective. (2) The observed difference δn between n and n' is applied as a numerical correction to all readings, i. e., $n = n' + \delta n$. The second method is to be preferred where the highest accuracy is required, since it will be found that many instruments, if adjusted to read correctly (to ± 0.0001) at one end of the scale, will have an error of ± 0.0003 or more at the other end. By calibrating the instrument with a set of standards, the appropriate correction, δn , can be applied to every region of the scale.

Dispersion Measurements.—The achromatization of the critical boundary furnishes a means of estimating the dispersion, $n_F - n_C$. It is evident that the greater the dispersion of the sample, the greater will be the necessary angle of rotation of the compensator. The amount of compensation is read on the so-called Z scale attached to the collar of the compensator (Fig. 11a, page 1204). The scale is marked arbitrarily with 60 equal divisions. The value of Z can be obtained to ± 0.05 division by "hunting" for the achromatic position,¹²⁹ i. e., oscillating the compensator with decreasing amplitude until the critical border is colorless. From Z the dispersion is obtained by:

$$n_F - n_C = A + kBS \quad (60)$$

¹²⁸ B. J. Mair, *J. Research Natl. Bur. Standards*, **9**, 461 (1932).

¹²⁹ L. E. Dodd, *Rev. Sci. Instruments*, **2**, 466 (1931).

where A and B are optical constants depending on n_D , given in tables, and k corrects for certain errors in each individual instrument. S is a function, given in tables, of angle σ (page 1205) through which the C prisms are turned; σ is found in tables from the reading Z .

It is essential, in using the Abbe refractometer for dispersion, to test the reliability of each instrument by making measurements on several standard substances covering a range of indices and dispersions. Many, especially the older instruments, are not supplied with reliable tables for converting Z readings to values of S and for finding A and B from n_D . Dodd¹²³ has shown that $n_F - n_C$ may be in error by up to 30% when the "blanket calibration charts" of A , B , and S are used without taking into account the characteristics of individual instruments. It is a laborious task to prepare¹²⁹ a corrected chart of A and B vs. n_D . For some instruments, adequate A , B , and S tables are given but a value for k is not; k becomes important if the compensator prisms happen to be mounted somewhat out of line. Tilton¹²⁵ has suggested that k can be conveniently found by using a hydrogen discharge tube as a light source for a calibration with substances of known dispersion. The dispersion, $n_F - n_C$, can be obtained to within 2% with the best instruments, provided they are used with proper light sources. The value, Z , by which the compensator is turned depends on the relative intensities of the various wave lengths in the source and, to a certain extent, on the limitations of the eye in judging color. Differences in $n_F - n_C$ as great as 100% can arise in measurements of the same sample with different light sources.¹²⁵ The instrument should therefore always be used with the light source for which it is calibrated.

Using monochromatic light, it is possible to measure n_λ to ± 0.0002 . With the prism clamped in the position of the n_D reading, the critical boundaries for various spectral lines are successively brought into coincidence with the cross-hair intersection by turning the compensator, and Z is read. The calibration and calculation^{125, 129} necessary for converting Z_λ and n_D into n_λ are elaborate and time consuming, and measurements of n_λ on the Pulfrich refractometer are much simpler and more accurate.

Precision Abbe Refractometers.—The Abbe-type instrument is in principle not well suited to better than 4th decimal accuracy for liquids, mainly due to the shielding of rays at grazing incidence by the auxiliary prism (see page 1212). Nevertheless, the need in industry for convenient refractometers having 5th decimal accuracy has stimulated attempts to improve the Abbe. The resulting new models do not fully meet the requirements, although they are valuable for particular applications when used with a consideration of the sources of error which become important at this level of accuracy.

The Bausch and Lomb "precision refractometer," shown in figure 12, is a modified Abbe instrument designed for an absolute accuracy of up to

2×10^{-5} .¹³⁰ It retains the simplicity and convenience of its prototype. An outstanding feature of this model, in contrast to the ordinary Abbe, is that it can easily be used to measure n for a variety of wave lengths (dispersion). Instruments are available with fixed ranges of n of 1.30 to 1.50, 1.40 to 1.70, and 1.33 to 1.64.

The improved accuracy is obtained by dispensing with the compensating prisms and by the use of unusually large and precise Abbe prisms mounted on a long, vertical, taper bearing. A monochromatic sodium light source is

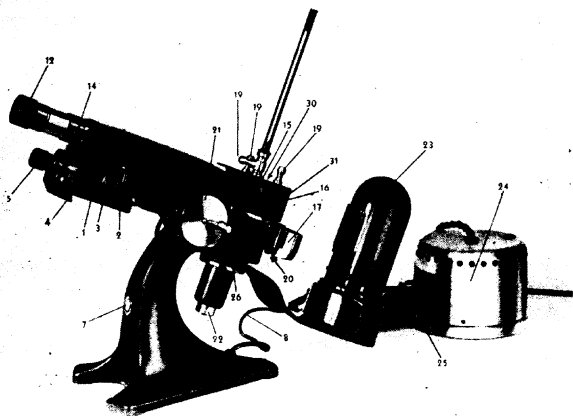


Fig. 12a.—Precision Abbe refractometer: 1, alidade; 2, reflector; 3, scale; 4, vernier; 5, scale magnifier; 7, scale switch; 8, scale lamp connector; 12, telescope; 14, knurled heads; 15, prism box; 16, light shield; 17, drip trough; 19, water connections; 20, drain connection; 21, hand wheel; 22, adjusting nut; 23, sodium Lab-Arc; 24, Lab-Arc transformer; 25, polarity plug; 26, lamp bracket lock screw; 31, prism hinge. Courtesy Bausch & Lomb Optical Co.

mounted with the instrument, and may be readily interchanged with other sources. The sector is marked in equal arbitrary divisions; and a table is used to convert scale readings to values of n_λ . The technique of measurement, described in detail in the Bausch and Lomb pamphlet,¹⁰⁷ is somewhat simpler than with the ordinary Abbe because the achromatization is eliminated. Otherwise, the operations are about the same as discussed above. A vernier reads directly to 0.01 division, corresponding to about 2×10^{-5} in n at the upper end of the scale and to about 6×10^{-6} at the lower end. With a good critical boundary, a setting of the cross hairs

¹³⁰ H. W. Straat and J. W. Forrest, *J. Optical Soc. Am.*, 29, 240 (1939).

may be reproduced within 0.005 division, the nearest half-hundredth mark being estimated. For such high precision, the temperature must be controlled to within $\pm 0.02^\circ$. A useful feature of the Bausch and Lomb precision Abbe refractometer is the auxiliary lens which can be moved into the telescope barrel to furnish a view of the liquid layer on the prism. In this

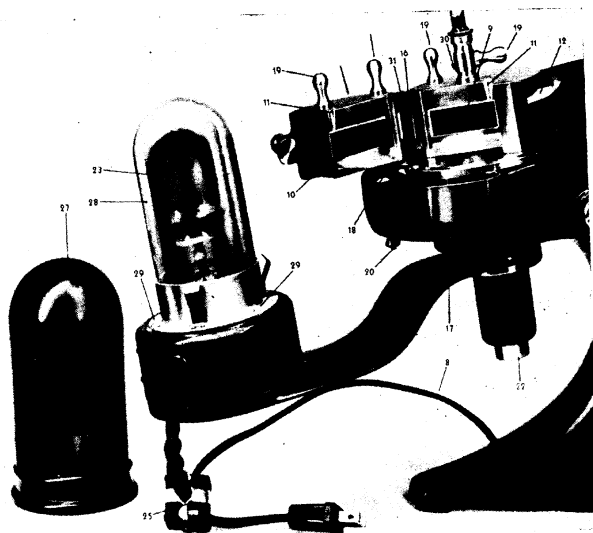


Fig. 12b.—Precision refractometer prism system: 9, working prism; 10, illuminating prism; 11, tubulation; 12, telescope; 15, prism box; 16, light shield; 17, drip trough; 18, drain plug; 19, water connections; 31, prism hinge. Courtesy Bausch & Lomb Optical Co.

way, air bubbles in the sample may be detected easily, or the interference fringes observed. This instrument is adapted to opaque samples, for which the refracting surface of the prism is illuminated internally through a prism window.

In working with a precision refractometer a few degrees above or below the calibration temperature, corrections for changes in prism index and in the state of the reference medium (air) become very important (see pages 1142 and 1196). These corrections can be calculated,¹²⁶ but, as Tilton¹²⁶ shows, it is more reliable to recalibrate the instrument with known standards.

Certain difficulties with the Bausch and Lomb precision refractometer should be kept in mind—(see 1, 2, 3, and 4 below)—since the potential accuracy of the instrument is not always realized in practice and even good reproducibility is obtained only under carefully standardized conditions. Cooperative tests have shown¹³¹ lack of agreement among different observers even in the 4th decimal; individual determination of n_D for five liquid samples at nine laboratories showed deviations from the average of 5×10^{-5} to 50×10^{-5} , with an average deviation of about 15×10^{-5} . Within a given laboratory the agreement among successive determinations was within 5×10^{-5} or better. One laboratory found differences of about 15×10^{-5} between reliable spectrometric measurements and determinations with a "Precision Refractometer" of three pure hydrocarbon standard liquids. In this case, the refractometer had been calibrated with glass test plates. Under the same conditions, the results for solid samples agreed with the spectrometric values within 2×10^{-5} , the inherent accuracy of the refractometer.

There are several features of the instrument which, if not fully appreciated during measurement, could be responsible for the above-mentioned discrepancies.

(1) *Shielding of Illumination.* The use of the auxiliary prism to confine and illuminate liquid samples necessarily results in the cutting off of those rays which are exactly at or close to the angle of grazing incidence (the light must enter the liquid at a finite distance from the surface of the refracting prism). Therefore, the true position of the critical boundary is actually invisible. The rays close to grazing incidence are low in intensity because they originate from a very small area of the refracting prism, along one edge. As a larger and larger area of this prism is illuminated, the intensity of rays still further from the critical angle increases and for a fully illuminated prism the apparent critical boundary is actually a rather broad, shaded region with the position of maximum intensity-change at considerably less than the critical angle. This results in the selection of some arbitrary point in the shadow as the critical boundary, depending on the particular observer, on the brightness, and on the position of the source. When three-fourths of the prism is illuminated, the width of the shadow corresponds to about 10×10^{-5} in n .

To minimize this effect, a shutter should be placed in front of the source so as to cut out most of the rays far from grazing incidence. This enhances the relative intensity of the close-to-critical rays. The edge of the rotatable lampshield is recommended as an adjustable shutter, or a slit can be placed over the source or over the face of the auxiliary prism. A

¹³¹ According to data of A.S.T.M. Committee D-2, Subcommittee XXV, kindly furnished by R. Matteson, California Research Corporation.

vertical slit 1×25 mm. at the source has been used successfully, although its position is critical; at the proper position, small displacements do not change the reading. In any case, the emergent face of the refracting prism should be observed by means of the auxiliary lens system while adjusting the shutter. When the proper illumination has been achieved, this face will be bright only at the extreme left of its area and will diminish toward the right. When about one-eighth of the surface is thus illuminated, the maximum uncertainty in the critical boundary position corresponds to about 1×10^{-5} in n .

The above source of error is not significant for solids since the distance of separation between sample and refracting prism is then very small. This gives support to the contention that measurement of liquids should be accompanied by calibrations with standard liquids, rather than with the glass test plate; the former procedure should be followed whenever there is any doubt as to the correctness of the illumination.

(2) *Shift in Prism Orientation.* Softening of the prism matrix by solvent action of the sample must be checked by frequent calibration,¹²⁶ especially for the 5th decimal work. A new matrix cement, Durisite, appears to minimize this difficulty. Concerning its use, the manufacturer of the instrument should be consulted. Other sources of accidental prism shifts have been excessive pressure on the snap-type lock on the prism case and loosening of the bearing nut, on early models. These defects have been remedied on later models.

(3) *Inadequate Protection of the Sample.* Evaporation during measurement can cause significant changes in composition and lowering of temperature.

(4) *Interference Bands.* The so-called "Herschel fringes,"¹³² parallel to the critical boundary, are more prominent in the Bausch and Lomb precision refractometer than in the ordinary Abbe refractometer because the auxiliary prism of the former instrument has a smooth instead of a mat surface. These bands should not interfere with adequate reproducibility if the observer is aware of the need for careful discrimination between the critical boundary and the sometimes brighter boundary of adjacent bands.

The Valentine improved precision refractometer¹³³ is an Abbe-type instrument adapted to white light; it allows a reproducibility of 2×10^{-5} and an accuracy of 5×10^{-5} in absolute values. The optical system is designed to eliminate haziness of borderline when reading solutions which tend to diffuse light.

Other Modified Abbe Refractometers.—In one model of the Spencer instrument the compensator prisms have been eliminated, allowing im-

¹²² See L. W. Tilton, *J. Research Natl. Bur. Standards*, **30**, 323 (1943), and page 1200.

¹²³ *Industro Scientific Co.*, Bellmore, Long Island, N. Y.

proved accuracy in dispersion measurements with monochromatic light sources. Both the O. G. Refractometer¹³⁴ and the Bausch and Lomb "Abbe-56" Refractometer have several mechanical improvements that allow for greater ease of operation—valuable in routine work. The latter instrument has the important feature of permitting an interchange of prisms by the operator, so that the range can be readily shifted; also, its prisms are mounted in the resistant Durisite cement (see page 1213).

C. DIPPING REFRACTOMETER

The dipping, or immersion, refractometer¹³⁵ is in principle very much like the ordinary Abbe but, since it has no auxiliary prism, is free from any inherent error due to shielding of grazing rays (p. 1212). It is designed for greater convenience and somewhat greater accuracy. A reading can be taken simply by immersing the attached prism in the thermostated sample and observing the position of the achromatized critical boundary on a scale in the eyepiece of the telescope. The dipping refractometer is used primarily for determining the concentrations of solutions, *e. g.*, in the sugar industry.¹³⁶ Calibration curves are established by measurements with known solutions. The refractive index can be obtained from scale readings by interpolation in tables furnished by the manufacturers, if a zero point correction is made. Browne and Zerban¹³⁶ and Gibb¹¹³ also give conversion tables. It is frequently stated that the accuracy of a determination under favorable conditions is about $\pm 3.5 \times 10^{-5}$ in n , corresponding to ± 0.1 scale divisions. It should be emphasized that ± 0.1 scale division is the *reproducibility* of each setting, while the *accuracy* of n is about $\pm 2 \cdot (3.5 \times 10^{-5})$ inasmuch as the value of n depends not only on the scale reading for the sample but also upon a second measurement with some standard substance in order to define a reference (zero) point on the scale; thus all measurements are differential. With a special eyepiece and under certain favorable conditions which depend on the nature of the sample, the illumination and the temperature control, the reproducibility of a setting may be made as high as ± 0.02 scale division.¹³⁶ The convenience of the ordinary dipping refractometer is somewhat offset by the difficulty of controlling the temperature, by lack of protection for the sample, and by the limited range of n . The range covered by each one of the six interchangeable prisms of the Bausch and Lomb instrument (~ 0.04 in Δn) is only slightly greater than that covered by the precision micrometer screw of the Pulfrich refrac-

¹³⁴ Gamma Scientific Co., New York.

¹³⁵ Manufactured by *Bausch & Lomb Optical Co.*, Rochester, N. Y., and by *Carl Zeiss, Jena (Ercon Corp.)*, New York, N. Y.).

¹³⁶ C. A. Browne and F. W. Zerban, *Physical and Chemical Methods of Sugar Analysis*. 3rd ed., Wiley, New York, 1941.

tometer. Moreover, the compensator readings are only a semiquantitative measure of the dispersion; in this respect the ordinary Abbe is superior.

The construction of the instrument and the path of the rays are shown in figure 13. The detachable prism, P , is mounted rigidly at the objective end of the telescope and, when dipped into the sample, allows the formation of a critical boundary in the usual way; an image of the critical boundary is formed within the telescope in the focal plane $F-F'$. The position of this image is located with reference to a micrometer scale engraved in equal arbitrary divisions on the focal plane of the objective, $F-F'$, that is, on the plane surface of the eyepiece lens, e_2 . A micrometer screw, S , allows the scale (lens e_2) to be shifted by known amounts, as read from the micrometer screw drum, D . In this way, the position of the critical boundary between two marks on the scale may be interpolated with higher accuracy than by direct estimation.

Zero Point.—In order to use the tables for converting readings to refractive index, the scale must be set to read properly when some standard substance (*e. g.*, water) is measured, or else a correction must be applied to subsequent readings. The latter method is recommended unless the discrepancy between measured and tabular value is large. To adjust the scale, the micrometer drum is loosened from the screw and reset to read correctly while the critical boundary is held in coincidence with the appropriate whole-division mark of the scale. Directions for loosening and resetting the drum differ from model to model and are usually given by the manufacturer. Browne and Zerban¹³⁶ describe the method for some models. The zero-point correction must be determined anew each time one prism is substituted by another.

Technique for Liquids.—The instrument is mounted on a frame above

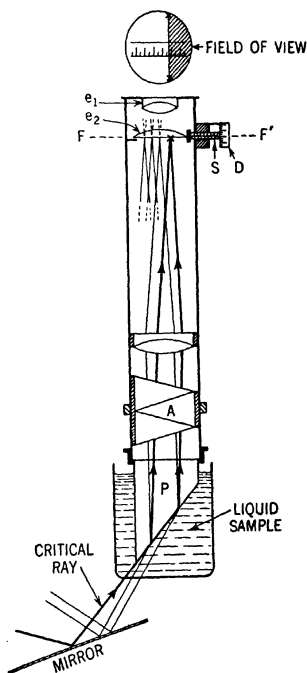


Fig. 13.—Dipping refractometer.

a water bath so that white light from a mirror below the (transparent) floor of the bath passes at grazing incidence into the completely immersed prism, P , after passing through a thermostated glass beaker containing about 15 ml. of sample. When temperature equilibrium has been reached, the critical boundary is made as sharp as possible by tilting the mirror or the instrument, by achromatizing with the compensator (A , Fig. 13), and by focusing the eyepiece lens, e_1 , on the scale, if necessary. A particularly sharp boundary can be obtained if a small mirror is placed directly below the sample and illuminated from above. A blurred or shifting boundary usually indicates poor temperature control. Some workers find it necessary to modify the standard bath by supplying it with circulating water from a thermostat ($\pm 0.05^\circ \text{C.}$). To avoid local cooling and other effects of evaporation, the liquid should be kept covered. Special cells are available which clamp tightly over the prism to protect the sample. The temperature within the beaker or cell should be measured each time. Between determinations, the prisms should be kept immersed in thermostated distilled water to minimize the time needed for reaching thermal equilibrium. The prism is quickly wiped dry with a soft cloth just before and after use. For small amounts (0.02 ml.), auxiliary prisms may be clamped below the refracting prisms, confining a thin layer of liquid as in the Abbe refractometer. In this case, the critical boundary is not at maximum sharpness, causing a lower accuracy.

Technique for Solids.—Solid samples are placed on the lower prism surface in the same way as described above for the Pulfrich and Abbe refractometers; and the same precautions apply.

Continuous-Flow Model.—Continuous analysis of a flowing liquid has been provided by mounting the prism of an ordinary dipping refractometer in a pressure-tight, thermostated cell. Pressures up to 80 lb. per in.² and temperatures up to 50°C. are permissible in the commercially available model.¹³⁷ The apparatus has been applied to plant control of butadiene purity.

3. Image Displacement Methods. The Spectrometer

The most direct method for determining refractive index and the simplest in principle is to measure the angles of incidence and refraction (Eq. 1 and Fig. 1), or, what is equivalent, to observe at a fixed distance from the sample the displacement of rays which pass through the sample at a fixed angle of incidence. Here the sample consists of or is contained within a prism.

Innumerable arrangements for this purpose have been devised, some of

¹³⁷ Precision Scientific Co., Chicago, Ill.

which are easily constructed in the laboratory. The simpler ones (see p. 1237) require only a distant illuminated slit as the source of incident rays, while for an accuracy of better than 10^{-3} the rays from a slit are collimated (rendered parallel) by a lens placed between the slit and the surface of incidence. Optical arrangements may be made for viewing the refracted image of the slit with the naked eye or with a telescope; it may also be recorded on a photographic plate.

Spectrometer.—The basic instrument in this field is the spectrometer. Its principal element consists of an accurately shaped prism mounted at the center of a movable graduated circle. A slit and collimator constitute a source of parallel rays having a known direction with respect to the prism surface. The image of the refracted source is located by means of a telescope mounted on the circle. The technique required has been described in detail by Guild¹³⁸ and by Kessler.¹³⁹

The spectrometer is a flexible instrument designed to meet the varying needs of spectroscopy and other branches of physics. It is not well adapted to routine work and such careful manipulations are required as to discourage its widespread use among chemists. However, there are some cases in chemical research for which this instrument is indispensable. Since any magnitude of angular deviation can be measured, there is no upper or lower limit to the refractive indices which can be covered. Thus it is possible to investigate certain fluorine compounds (*e. g.*, $n\text{-C}_7\text{F}_{18}$, $n_D^{30} = 1.2572$) which are below the range of standard refractometers. Also, observations on the less intense spectral lines can be made, which is important for complete studies of dispersion. Investigations of the ultraviolet and infrared regions of the spectrum are made possible by fitting the spectrometer with appropriate lenses, *e. g.*, quartz. In refractometry the spectrometer is used primarily for solids which must be measured with extreme accuracy (1×10^{-6}) or which must be carefully oriented (*e. g.*, anisotropic crystals). By using a hollow prism, formed, for instance, by two windows attached at an angle to the ends of a tube, it is also possible to obtain very accurate values of n for liquids. Tilton and Taylor¹⁴⁰ have secured probably the best available refractometric data on water in this way; they have described the apparatus, including the means of temperature control.

The least expensive spectrometer on the market is capable of an accuracy of about 2×10^{-4} in n (1 minute of arc). Satisfactory models may

¹³⁸ R. Glazebrook, *Dictionary of Applied Physics*. Vol. IV, Macmillan, London, 1923 pp. 760–772.

¹³⁹ H. Kessler, in *Handbuch der Physik*. Vol. XVIII, Springer, Berlin, 1927, pp. 632–682.

¹⁴⁰ L. W. Tilton and J. K. Taylor, *J. Research Natl. Bur. Standards*, 20, 419 (1938).

be purchased from almost any of the well-known firms specializing in precision optical equipment. Hollow prisms for liquids are also available.¹⁴¹

Various spectrometer-like instruments with photographic means of locating the refracted rays, especially important for the invisible region of the spectrum, have been described.^{140, 142-144}

Differential Instruments.—The image displacement principle has been incorporated into a number of useful differential refractometers. They have in common the characteristic that their refracting surfaces are made up of the equivalent of two adjacent prisms, one containing the liquid sample (n), the other consisting of or containing a reference substance (n_0). As a rule the optics are designed to provide for zero deflection of an image when the two substances have equal refractive indices and a deflection nearly proportional to $(n - n_0)$ when they are different.

These instruments have an important place because they allow direct comparisons with relatively high accuracy and at the same time require only simple technique and apparatus. They can be assembled on an optical bench from standard optical parts. Furthermore, the differential principle overcomes the usual difficulty with temperature control encountered in precision spectrometry. As mentioned by Dutton¹⁴⁵ and by Holmes,¹⁴⁶ it is possible to automatically record the position of the displaced image and thus provide for continuous measurements (see p. 1234), or to project the image on a translucent scale, giving an instantaneous indication of refractive index.

A good account of the many early refractometers based on the above principle is given by Kessler¹⁴⁷ who also derives the basic equations. More recently, descriptions of various modifications have been published; for 6th decimal accuracy the reader is referred to the papers of Debye¹⁴⁸ and of Brice and Speiser.¹⁴⁹ For this high accuracy the range is limited to about 0.01 in $n - n_0$. The simple apparatus of Holmes has an extended range of 1.47–1.65 and is accurate to ± 0.001 ; a minor change allows ± 0.003 in the usually inaccessible range 1.61–2.10.

The differential refractometer of Debye has been applied in connection with the estimation of large molecular weights from data on light scattering and refractive index of dilute solutions. The apparatus (Fig. 13a) is

¹⁴¹ For example, *Klett Mfg. Co.*, New York; *C. F. Whilems Ltd.*, Essex, England.

¹⁴² K. Feussner, *Z. Physik*, **45**, 689 (1927).

¹⁴³ H. Voellmy, *Z. physik. Chem.*, **127**, 305 (1927).

¹⁴⁴ H. Martens, *Ann. Physik*, **6**, 603 (1901).

¹⁴⁵ H. J. Dutton, *J. Phys. Chem.*, **48**, 179 (1944).

¹⁴⁶ J. G. Holmes, *J. Sci. Instruments*, **22**, 219 (1945).

¹⁴⁷ H. Kessler, in *Handbuch der Physik*. Vol. XVIII, Springer, Berlin, 1927, p. 668

¹⁴⁸ P. P. Debye, *J. Applied Phys.*, **17**, 392 (1946).

¹⁴⁹ B. A. Brice and R. Speiser, *J. Opt. Soc. Am.*, **36**, 364 (1946).

essentially a spectrometer employing a prism of the solution immersed in a rectangular cell containing the solvent. An adjustable slit, S , of good quality, is illuminated by monochromatic light, obtained, for example, by filtering the light from a mercury lamp, O , of the AH4 type; a parallel beam formed by lens L_1 passes through rectangular glass cell C (containing the solvent) in which is suspended the hollow prism glass cell, P , for the solution. The refracting angle of the prism is about 125° . Lens L_2 forms an image of the slit in the field of a filar micrometer, M , at a distance of about 190 cm. from the entrance slit. The position of the image is noted when both prism P and cell C contain the solvent; on introducing the solution into P , the image is displaced by an amount proportional to $n - n_0$, if the difference is not too large. For the instrument described,

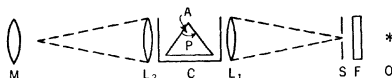


Fig. 13a.—Differential refractometer of P. P. Debye.¹⁴⁸

full-scale deflection on the micrometer scale is still within the linear range.

For small image displacements, the displacement, x , is:

$$x = 2f \tan (A/2)(n - n_0)$$

where f is the focal length of L_2 and A the refracting angle of prism P . The deflection for fixed f and A depends only on the difference of refractive index between solvent and solution, and not on the absolute value of that of the solvent; hence the instrument can be calibrated by means of a suitable system, for example sucrose-water solutions, and then used for any other solvent-solute system.

For temperature control the cells are placed in a jacket through which water is pumped at a temperature regulated by a good enclosed type of mercury regulator.

Schlieren Technique.—A specialized but sometimes indispensable technique is the Foucault-Toepler Schlieren method, developed primarily as a means of following the moving boundary in electrophoresis experiments. Any problem involving the observation of sharp concentration gradients in liquids may benefit from this method, recently made convenient by Longworth.¹⁵⁰ See chapter XII for a description of this and related means of determining refractive index gradients. Schlieren is only indirectly related to the previously described image displacement methods. It depends upon the formation of shadows on the image of a cell by the inter-

¹⁵⁰ L. G. Longworth, *Ind. Eng. Chem., Anal. Ed.*, **18**, 219-229 (1946).

ception of convergent rays which, while passing through the cell, have been deviated by any inhomogeneous (prism-like) layers of liquid within the cell. To intercept these rays, a sharp-edged "Schlieren diaphragm" is gradually moved into the optical path. The position of a shadow on the photographed image can be correlated with position x in the cell of the concentration gradient giving rise to this shadow. The corresponding refractive index gradient, dn/dx , is proportional to the distance of the Schlieren diaphragm edge from the focal line of the convergent rays.

Longworth also describes auxiliary hollow-prism cells which allow differential refractive index and dispersion measurements of liquids to $\pm 1 \times 10^{-6}$ with Schlieren apparatus. The complete apparatus is available commercially.¹⁵¹

4. Interferometric Methods

The interference of light waves has been extensively applied to very accurate measurements of differences in index of refraction. Two intersecting light beams from the same source are made to produce a set of "interference bands" in the plane of intersection, which is viewed with a lens. If a transparent substance of index n is placed in the path of one beam and another substance of equal length but different index, n_0 , in the path of the other beam, there is a displacement of the interference bands. The interferometer allows one to determine the difference, $n - n_0$, by measuring the shift in position of the interference bands.

The ordinary commercial instruments have a maximum accuracy of about $\pm 5 \times 10^{-7}$ in Δn for liquids when white light is used. With monochromatic light, an accuracy of $\pm 1 \times 10^{-7}$ can be obtained and with certain modifications (see page 1231) in the instrument, of $\pm 1 \times 10^{-8}$. The range which can be covered is inversely proportional to the accuracy; it can be adjusted by placing the sample in cells of various length. Thus, when an accuracy of $\pm 5 \times 10^{-7}$ is required, the maximum difference which can be measured is about $(\Delta n)_{\max.} = 0.0006$, using a 4-cm. cell. Increasing the range to $(\Delta n)_{\max.} = 0.05$ by using the shortest practicable cell (1 mm.) causes the error to increase to about $\pm 4 \times 10^{-6}$. Certain modifications of technique and appropriate monochromatic sources permit measurement of the dispersion $(n_{\lambda_1} - n_{\lambda_2})$ or of n_{λ} relative to a standard (page 1230).

An important advantage of the interferometer is that, because it is a differential instrument, the requirements of temperature control are not nearly as exacting as with the previously mentioned refractometers. Also, the range is unlimited as long as reference fluids are available within the limits $n - n_0 = \pm 0.05$. The interferometer is the most convenient, as

¹⁵¹ Klett Mfg. Co., New York.

well as the most accurate, instrument for measuring the apparent molar refraction of solutes in dilute solutions, or $\Delta n = n_{\text{soln.}} - n_{\text{solv.}}$. However, with organic compounds, the error due to impurities will ordinarily be greater than the error of the measurement, unless the compounds are prepared with special care. On the other hand, there are many problems in which the interferometer is invaluable as a means of analyzing solutions or of observing small changes in concentration. Thus, for example, one can continuously follow the *rate* of a reaction without removing samples. The purity of a substance may be checked rapidly by a comparison with a standard, or by comparing successive portions obtained by distillation or recrystallization. A rapid technique for the determination of concentration of protein in blood serum has been based on the use of the interferometer.¹⁵² As little as 0.002% excess D₂O in water can be detected with an ordinary instrument if a large sample is available.

A. OPTICAL PRINCIPLES

The principle of the interferometer is explained by the simplified model illustrated in figure 14. A converging lens, *L*, is placed in the path of two

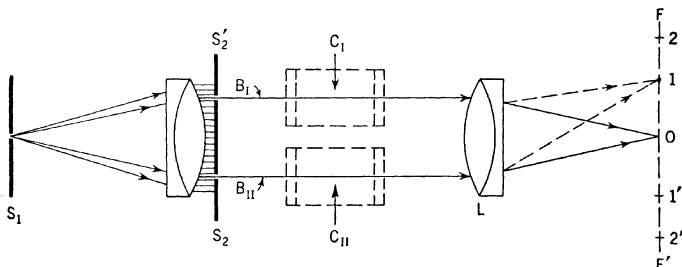


Fig. 14.—Simplified interferometer (slits S_1 , S_2 , and S_2' refer to the openings in the heavy lines).

narrow parallel beams, B_I and B_{II} , defined by the secondary slits, S_2 and S_2' , of monochromatic light coming from the same source. An image of the source, primary slit S_1 , is formed in the focal plane $F-F'$ of L , which can be viewed with an eyepiece placed in front of $F-F'$. If the primary slit, originally large, is made more and more narrow, the appearance of the image changes. It finally consists not of a single image of S_1 but of alternate dark and light bands, equally spaced and approximately equal in width (see

¹⁵² P. Hirsch, in E. Abderhalden, *Handbuch der biologischen Arbeitsmethoden*. Section II, Vol. II, Urban & Schwarzenberg, Berlin and Vienna, 1926, p. 761.

Fig. 15b). This interference pattern is caused by the alternating reinforcement and cancellation of the intersecting wave fronts in the focal plane.

Figure 15a shows the effect of the intersection of monochromatic wave fronts which require different lengths of time to reach the same point in the focal plane, *i. e.*, which travel different optical path lengths between the time of their separation at the source and their reunion in the plane $F-F'$. At some points in this plane the wave crests of one ray are superimposed on the troughs of the other; at other points, crest is superimposed on crest.

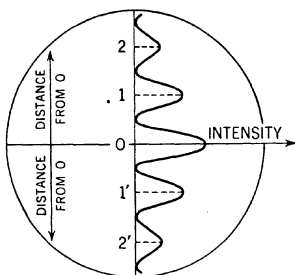


Fig. 15a.—Distribution of light in the interference pattern, with monochromatic light and finite slit width.

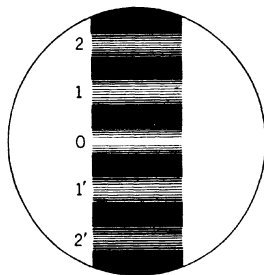


Fig. 15b.—Appearance of interference bands with monochromatic light and finite slit width. Ruled areas represent regions of continuously decreasing intensity on either side of the central maximum.

At still other points, the relation between crests and troughs is intermediate. When the waves are completely out of phase, *i. e.*, the crests of one coincide with the troughs of the other, absence of light results (see points midway between 0 and 1, 1 and 2 in figure 15a).

The condition for minimum (zero) light intensity at a point in the focal plane is:

$$P = (N + \frac{1}{2})\lambda \quad (61)$$

and for maximum intensity:

$$P = l_{II} - l_I = N\lambda \quad (62)$$

P is the *optical path difference* in centimeters. N is an integer called "band order," and l_I and l_{II} refer to the optical path length traversed by the two rays. The optical path length is defined as $l = \sum n_i d_i$, where n_i and d_i refer to the refractive index and thickness, respectively, of the i th medium traversed by the light ray; l is thus equal for two paths for which the light

requires the same time to traverse. λ refers to the wave length in centimeters.

For infinitely narrow secondary slits (S_2, S'_2) there exists one line in focal plane $F-F'$ for which $N = 0$, i. e., $l_{II} = l_I$ (0 in Fig. 14). Accordingly, 0 is a position of maximum light intensity. The latter falls off rapidly on either side of 0 to reach a minimum at a distance corresponding to $P = \lambda/2$. At a still greater distance from 0, corresponding to $P = \lambda$, the intensity reaches a maximum again (1, 1', Fig. 14), etc. Since slits S_2 and S'_2 actually have a finite width, instead of lines there will be regions¹⁵³ of maximum intensity, and the interference pattern consists of alternate dark and light bands with rather sharp edges (Fig. 15b). A minimum corresponding to $P = (N + \frac{1}{2})\lambda$ is called N th order minimum, the bright band corresponding to $P = N\lambda$, " N th order maximum," the central maximum ($N = 0$) the 0 order band. The distance, x , between the maxima is approximately proportional to the wave length of the light used (i. e., $x_1 \approx k\lambda_1$).¹⁵³

With polychromatic light, there is a different set of bands for each λ and at certain intervals the bands of one set overlap with those of another. Consider, for example, that just two wave lengths, λ_1 and λ_2 , are present in the source, with $\lambda_2 = \frac{1}{2}\lambda_1$. Then, assuming $x_1 = k\lambda_1$, $x_2 = k\lambda_2$, $x_2 = \frac{1}{2}x_1$. Hence, in the region near the 0 order band, a band from the λ_1 set will be superimposed on a λ_2 band at intervals of $5x_2$ centimeters. The result is illustrated in figure 16, where, if $\lambda_1 = \text{yellow}$ and $\lambda_2 = \text{blue}$, every ninth band (S) will be green.

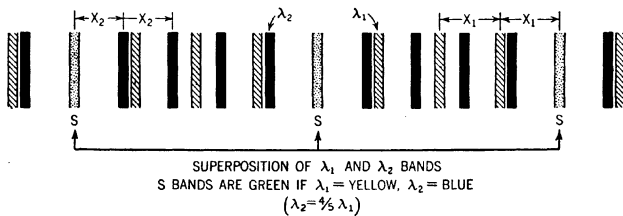


Fig. 16.—Overlapping of colored bands (interferometer).

When white light is used, as in many applications of the interferometer, only the 0 order band is white because only here are all wave lengths superimposed. It is bordered by a sharply defined black region. All other bands will be colored (Fig. 17) due to unequal spacing of the maxima for the various wave lengths. Only the immediate neighbors of the 0 order band are fairly distinct: Since $\lambda_{\text{blue}} < \lambda_{\text{red}}$, they are fringed with blue on the side nearest the central band, and with red on the

¹⁵³ See, for example, W. E. Williams, *Applications of Interferometry*. Methuen, London, 1930.

other side. However, even a short distance from 0 the overlapping becomes so uniform that only a few distinct bands are observed.

If in each beam of figure 14 are placed identical transparent air-filled chambers, C_I and C_{II} , no change in the interference bands will occur. A reference line is now selected in the focal plane, for example, 0 in figure 14, by means of a cross hair rigidly fixed in the eyepiece so that its image is superimposed on the edge of one of the bands, the 0 order band, for instance.

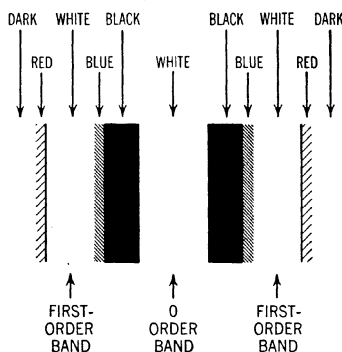


Fig. 17.—Appearance of interference bands with white light

By gradually replacing the air, n_1 , in one of the chambers by some gas of index n_2 ($n_2 > n_1$), the bands will slowly move. After a while, the first-order band will reach the position defined by the cross hair, to be followed by the second-order band, and so on. Eventually the lower order bands pass out of the field of view; and by the time the one chamber has been completely refilled, an N th order band at, or near, the cross hairs is being viewed. By counting the number of bands (ΔN) which correspond to the change (ΔP) in the optical path difference at 0, the difference in index ($n_1 - n_2$) between the unknown gas and the air or another reference substance can be calculated. Differentiation of equation (61) yields the relation between change in path difference and change in band order at any point:

$$\Delta P = \lambda(\Delta N) \quad (63)$$

If both beams pass through a thickness, d of each gas it follows from equation (62) and from the definition of optical path length (see page 1222) that:

$$\Delta P = n_1 d - n_2 d \quad (64)$$

Substituting in Equation (63):

$$n_1 - n_2 = \frac{\lambda}{d} (\Delta N) \quad (65)$$

This method is, in principle, actually used in measuring $n_1 - n_2$ for gases. However, for liquids, the number of bands which go past the reference mark cannot be counted because the introduction of the sample is necessarily discontinuous. Nor is it possible to determine the order of interference by merely looking at the bands, since, with monochromatic light, all bands look almost alike. A number of indirect methods of determining the

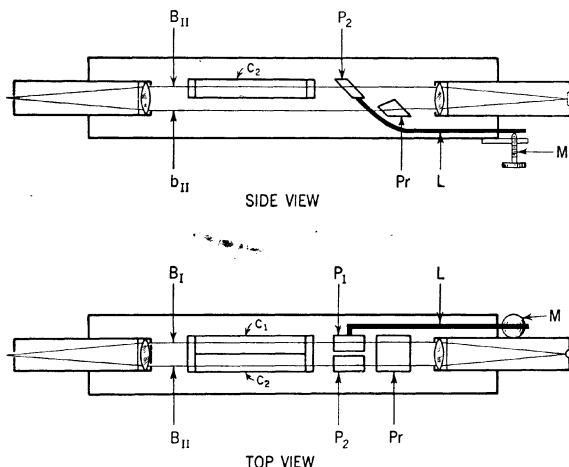


Fig. 18.—Rayleigh-Haber-Löwe interferometer.

band order have been developed.¹⁵⁴ The simplest, though not always the most reliable, method depends on "labeling" the 0 order fringe by the use of white light. The position of the achromatic (0 order) band is observed; then the sample is introduced, whereupon this band can no longer be seen because it has passed out of the field of view. The achromatic band can be brought back to its original position by continuously changing the optical path length in one of the beams; this is conveniently done by tilting a glass plate in the path of one beam until the effective thickness of the plate is great enough to compensate for the presence of the sample in the path of the other beam. The amount by which the compensator plate is turned is a

¹⁵⁴ See, for example, W. Geffcken, *Z. Elektrochem.*, **37**, 233 (1931).

measure of the difference in index, $n_1 - n_2$. It is evident from equation (65) that smaller values of $n_1 - n_2$ can be measured by increasing the cell length.

B. RAYLEIGH-HABER-LÖWE INTERFEROMETER FOR LIQUIDS

The models of this instrument which have been manufactured by Baird,¹⁵⁵ Hilger, and Zeiss differ only in small details. The essential parts are shown in figure 18. The optics are nearly the same as those discussed for the simplified case of figure 14. However, instead of cross hairs, an auxiliary set of interference bands is used as the frame of reference. This is accomplished

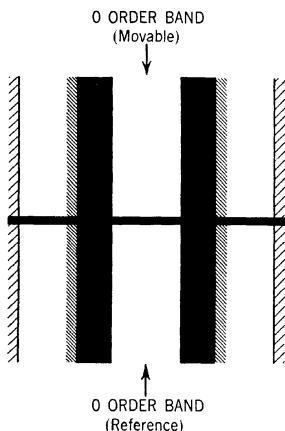


Fig. 19.—Center of field of view at coincidence, Rayleigh-Haber-Löwe interferometer.

by equally dividing each of the two beams, B_I and B_{II} (Fig. 14), so that four beams, b_I , b_{II} , B_I , and B_{II} , result. The lower beams, b_I and b_{II} , traverse only air and the optical parts including the lifting prism, Pr , and form the auxiliary reference set of vertical bands in the lower half of the field of view. Each upper beam, B_I and B_{II} , passes through a cell, c_1 , c_2 , and a plane-parallel glass plate, compensator plate P_1 , P_2 . This pair forms a set of bands above the reference pattern, as in figure 19. Prism Pr serves to raise beams b_I and b_{II} toward B_I and B_{II} so that only a fine dark line separates the two sets of bands in the field of view. By changing the orientation of P_1 relative to fixed plate P_2 , the optical path length of beam B_I can be increased or decreased. Thus, the upper set of bands can be moved relative to the fixed auxiliary set by turning micrometer screw M which, through lever L , transmits a slow tilting motion to P_1 . The amount of tilting is measured on the micrometer screw scale. The effective thickness of P_1 becomes very great when the plate is nearly parallel to the light beam, and changes considerably for a very small tilting. However, when the plate is perpendicular to the beam, the effective thickness is very insensitive to a given displacement of the micrometer screw.

To measure Δn on this type of instrument, one ordinarily uses the compensation method described on page 1225. When white light is used,

¹⁵⁵ Baird Associates, Inc., Cambridge, Mass.

the value of Δn corresponds closely to that which would be obtained by using sodium D light (for white light, *average* $\lambda = 5600 \text{ \AA.}$, and for sodium D light, $\lambda = 5893 \text{ \AA.}$). The micrometer scale readings are usually calibrated in terms of concentration.

A three- to six-volt tungsten filament bulb with rheostat to vary the intensity is used as the light source for some models. For others, a "Pointolite" tungsten source (see Chapter XXI) is employed. Too great an intensity illuminates the background and reduces contrast at the band edge. The corresponding parts of the two sets of bands should be of the same brightness, the separating line narrow and sharp (Fig. 19). If these conditions are not satisfied most probably the tungsten filament is not oriented properly with respect to the vertical primary slit; it should cross the slit at right angles so that a point source results. Under optimum conditions, the central white bands will show sharp vertical boundaries. A deviation from the vertical may be corrected by rotating the eyepiece.

One should be able to set the two band systems in coincidence within about $\pm 1/20$ of a band width, $1/20$ of a band corresponding to about 0.05% of $(\Delta n)_{\text{max}}$. The accuracy of the settings can vary considerably from one observer to another due, for example, to a slight color blindness; thus each person should make his own calibration of the instrument.

The cell length to be used depends on the accuracy and range required, as well as on the amount of sample available. In the "portable" instrument, which is especially compact and rugged, a mirror system causes the light beams to pass twice through the sample. Thus, the effective length of the cell is doubled. Even with the shortest practicable cell (1 mm.), the accuracy of about $\pm 4 \times 10^{-5}$ in Δn is about as great as for the Pulfrich or the dipping refractometer, and the procedure is considerably more convenient. Each individual cell should be marked, for no two cells have exactly the same length. A separate calibration is required for each pair of cells.

The cells are obtainable in two forms, as gold-plated metal troughs with cemented windows and as fused all-glass cells without cement. The latter are essential for many organic liquids. Occasionally, they have minute holes near the windows which can cause serious errors without otherwise being apparent. It is therefore advisable to test each cell by filling it with silver nitrate solution and allowing it to stand a few hours in a solution of sodium chloride. A dye can also be used as a detector of imperfections. The usual cells are unsatisfactory for hygroscopic liquids or solutions with very volatile components. Bruins and Cohen¹⁵⁶ suggest a tight-fitting

¹⁵⁶ E. Bruins and H. K. Cohen, *Z. physik. Chem.*, **103**, 337 (1923).

brass plug for the chamber opening. Bartell and Sloan¹⁵⁷ describe a cover with a mercury seal.

The two cells, containing sample and reference solution, respectively, must be at the same temperature within very close limits. To measure Δn to 2×10^{-7} , the difference must not be more than about 0.002°C . Temperature gradients within a cell, or excessive differences between the two cells, will cause the interference bands to have a wavy, indistinct appearance. This is a frequent source of error. On the other hand, measurements with the interferometer are not greatly influenced by changes in the absolute value of the temperature; it usually suffices to keep the bath temperature within about 0.5° of that of the calibration. The per cent error, E , in Δn per degree C. is given by:

$$E = \frac{d(\Delta n)}{dt} \cdot \frac{1}{n} = \frac{r_1 - r_2}{t_1 - t_2} \cdot \frac{100}{r_1} \quad (66)$$

where r_1 and r_2 are the scale readings at t_1 and t_2 degrees C. E is usually about 0.2% per degree.

The Baird and Hilger instruments should be placed in a thermostated box since it has no jacket around the cells. For the Zeiss instrument, it is usually necessary to make special provision for stirring the water bath which comes in contact with the cells.¹⁵² With long cells it may also be necessary to stir the liquids themselves. In this way, temperature equilibrium is reached within a few minutes. Since the light passes through a considerable thickness of thermostat water, the bath should be kept free of turbidity by frequent renewal or a decrease in band intensity results. The bath liquid must have about the same refractive index as that of the sample, if the cell windows are not exactly parallel¹⁵⁸; e. g., for $n > 1.42$, a n -dibutyl phthalate ($n = 1.49$) bath would be preferable to water ($n = 1.33$). Volatile liquids often cause cooling by evaporation, resulting in wavy or blurred bands. In this case, the cell should be provided with a special cover.

Comparison of Liquids. White Light.—With the reference liquid in both chambers, the adjustments for obtaining sharp straight achromatic bands are made (see p. 1227). The micrometer screw is turned until the upper achromatic band exactly coincides with the reference band, as in figure 19. The mean of several such readings, which will have a value of about 1 or 2, gives the zero point, r_0 . The latter should be checked daily or whenever a different pair of cells is used.

Cell c_1 (Fig. 18) is emptied and refilled with the unknown sample after

¹⁵⁷ F. E. Bartell and C. K. Sloan, *J. Am. Chem. Soc.*, **51**, 1637 (1929).

¹⁵⁸ F. Löwe, *Fortschritte der chemischen Technologie in Einzeldarstellung*. Vol. VI, Steinkopf, Dresden, 1925, p. 155.

thorough rinsing. The micrometer screw is turned until the bands again coincide. The setting is repeated several times, and the corresponding mean scale reading, r is obtained. The difference $\Delta n = n - n_0$ or $\Delta c = c - c_0$ is obtained from $r - r_0$ by means of an appropriate calibration curve.

A typical calibration curve for concentration determinations is shown in Fig. 20. Either c or c/r may be plotted¹⁵⁹ for a number of known solutions against $r - r_0$. At certain intervals, the plot may show discontinuities of about 20 scale divisions (1 band). In the region of a discontinuity (Fig. 20, dotted lines), alternative values of c are obtained for a given $r - r_0$, because the curve segments overlap. The discontinuities are caused by the fact that it is not always possible to locate the 0 order band unambiguously. It becomes difficult to distinguish the latter from the first-order band when the optical dispersion of solution plus glass plate P_1 differs too much from the dispersion of reference liquid plus P_2 . If this difference in

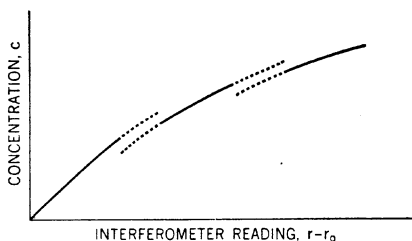


Fig. 20.—Typical calibration curve of interferometer.

dispersion were continuously increased from zero, the edges of the 0 order band would gradually become colored, while one of the adjacent first-order bands would lose its usual colored fringes and become white. The other first-order band would at the same time acquire broader color fringes and appear more like the original second-order band. Thus the location of the 0 order band will be in error by one whole band width because the reference set remains, of course, unchanged. For still greater differences in dispersion, the adjacent second-order band becomes white, and so on. The apparent shift of the 0 order band is a consequence of using white light: If the optical dispersions of the two beams are different, not all wave lengths will be exactly superimposed in the position of the 0 order band and an overlapping with the complementary colors of an adjacent fringe takes place (see page 1223).

The insidious error arising from this "dispersion effect" can be minimized

¹⁵⁹ See L. H. Adams, *J. Am. Chem. Soc.*, **37**, 1181 (1915).

by limiting the measurements to small concentration differences. With substances of low dispersion, it is usually safe to make comparisons as long as the scale readings are not greater than about one-third of the maximum range. Aliphatic compounds show, in general, a much smaller dispersion effect than do aromatic compounds. For example, Macy¹⁶⁰ reports that certain aliphatic compounds in water show no break in the calibration curve up to one gram per liter while, for the same cell and solvent, phenylurethane shows a break at about 200 milligrams per liter. Similarly, compounds with conjugated double bonds, with halogen substituents, or with other easily polarizable groups, can be expected to give the "dispersion effect." The shift in the achromatic band hardly ever occurs for $r - r_0 < 1/15$ of its maximum value.¹⁵⁹ It is therefore nearly always possible to obtain reliable results by comparing the unknown solution directly with a known solution of slightly lower or higher index. In a limited range, it is permissible to make a linear interpolation for c (that is, assume $r - r_0 = kc$). If, during a measurement, there is any doubt as to the identity of the 0 order band, the comparison should be repeated with a reference solution closer to the unknown. In this way, very concentrated solutions may be compared; but it is usually tedious to prepare known standard solutions which are sufficiently close to the unknown to exclude the dispersion effect.¹⁶¹ When the full range of the instrument is required or when many routine measurements are to be performed, the calibration method of Karagunis, Hawkinson, and Damköhler¹⁶¹ is recommended. These authors label the 0 order band so that it can be identified even though colored. For a given solute and solvent, with known dispersions, it is possible to specify a set of optical constants of the compensator plate which would eliminate the discontinuities in the calibration curve.¹⁶² Thus a different compensator could be installed for each type of routine analysis.

Monochromatic Light. Dispersion.—The use of monochromatic light becomes necessary whenever an accuracy of better than about 0.1% in Δn is required. A discussion of the technique for careful measurements of Δn with monochromatic light is given by Brodsky and Scherschever,¹⁶³ by Prang,¹⁶⁴ and by Geffcken and Kruis.^{47, 56, 57}

In its present form, the interferometer is not very convenient for measuring relative dispersion, especially since monochromatic light sources of very high purity are required to prevent "overlapping." The line spectra

¹⁶⁰ R. Macy, *J. Am. Chem. Soc.*, **49**, 3070 (1927).

¹⁶¹ G. Karagunis, A. Hawkinson, and G. Damköhler, *Z. physik. Chem.*, **A151**, 433 (1930).

¹⁶² Y. Doucet and J. Desfretière, *Compt. rend.*, **224**, 337 (1947).

¹⁶³ A. E. Brodsky and J. Scherschever, *Z. physik. Chem.*, **A155**, 417 (1931).

¹⁶⁴ W. Prang, *Ann. Physik*, **31**, 681 (1938).

of the alkali metals, and the λ 5460.7 line of the mercury arc in conjunction with filters are most suitable. (For a further discussion of light sources, see Kruis.⁵⁶) The usual, rather laborious, method consists in finding the order of interference for each wave length in the following steps: (1) With pure solvent in the cells, the zero point reading, r_0 , is determined with white light in the usual way. (2) With solvent replaced by solution in one cell, using white light, the compensator plate is turned until the achromatic bands coincide. This approximately locates the 0 order band. Monochromatic light is substituted, keeping the compensator fixed. The monochromatic 0 order bands will now be the ones nearly at coincidence; the 0 order band of the movable set is then located more exactly by carefully adjusting to coincidence and noting the corresponding scale reading, r . (3) The micrometer is slowly turned toward r_0 , the number of bands passing being counted until, at scale reading r' , coincidence is obtained between bands nearest to r_0 . The position of the nearest band (r') can be determined unambiguously by setting the compensator at r_0 , then turning the screw by the minimum amount required to bring about a coincidence; the scale reads r' . The number of bands, N , counted between r and r' is the value of (ΔN) , to the nearest whole number, *i. e.*, $(\Delta N)_\lambda = N \pm f$, where f is a fraction less than 0.5. (4) The fractional part of a band, f , can be determined from the difference, $r_0 - r'$, by measuring for each wave length the conversion factor k_λ , the number of scale divisions which are equivalent to the separation between corresponding parts of adjacent bands. Each k_λ must be determined in the neighborhood of reading r_0 since ΔN is not an exactly linear function of r . Then $f = (r_0 - r')k$. The fraction, f , is added to or subtracted from N , depending on whether r' is larger or smaller than r_0 . Equation (65) in the form:

$$(n_\lambda - n_{0\lambda}) = \frac{\lambda}{d} (\Delta N) \quad (67)$$

is then applied to each wave length, which allows the calculation of n_λ if $n_{0\lambda}$ of the reference liquid is known, or allows the calculation of $n_{\lambda_1} - n_{\lambda_2}$ if $n_{0\lambda_1} - n_{0\lambda_2}$ is known. This method is, of course, also subject to the errors caused by the "dispersion effect." For recent improvements in the use of the interferometer for accurate dispersion measurements, see below.

C. MODIFICATIONS OF RAYLEIGH-HABER-LÖWE INTERFEROMETER

Kruis and Geffcken^{47, 56, 57} have described precautions and refinements in the usual instrument necessary to obtain an accuracy of 1×10^{-8} in Δn , using monochromatic light. Methods of temperature control are given special consideration.¹⁶⁵ An ingenious modification of the Rayleigh

¹⁶⁵ See also N. F. Hall and T. O. Jones, *J. Am. Chem. Soc.*, **58**, 1917 (1936).

interferometer by Geffcken^{45, 55} eliminates the dispersion effect by using monochromatic light in conjunction with a "rotating chamber." The band order is determined by counting the number of bands which pass while the cells are slowly turned through a given angle with respect to the *axis of the interferometer*. It is unfortunate that this chamber device is not yet available commercially, for with it one would be able to make rapid precision measurements of even very large differences in n for monochromatic light and also to measure the relative dispersion more conveniently. Measurements of the absolute value of refractive index of a liquid substance may also be made with the rotating chamber, but the experimental details have not been published.⁵⁵⁻⁵⁷

The Rayleigh-Williams interference refractometer,¹⁶⁶ with quartz optics, promises to be very useful in refractometric work because it allows accurate and rapid measurement in the whole range from 8000 to 2000 Å. Using a spectrograph in conjunction with the Rayleigh-Williams interferometer, one can at once make a photographic record of the dispersion for all spectral lines, using an appropriate single light source. The combination of an interferometer with a spectrograph is known as a *spectral interferometer*. The quartz optics makes possible the investigation in the ultraviolet, *e. g.*, of compounds which are strongly colored in the visible region of the spectrum. A spectral interferometer of especially high accuracy by Geffcken and Kruis⁴⁸ resembles in some respects the Rayleigh-Williams instrument.

Application to Crystals.—The Rayleigh interferometer has been adapted to the measurement of the refractive index of crystals (n to $\pm 3 \times 10^{-5}$).⁵⁹ For isotropic solids, the technique is very simple. The ordinary interferometer cells are replaced by a single vessel having parallel windows which are large enough to allow both beams to pass. The vessel is filled with an appropriate liquid whose index of refraction can be varied continuously. A single crystal fragment as small as 0.5 millimeter in cross section is immersed in the liquid and placed in the path of one of the beams. If $n_{\text{liq.}}$ differs slightly from n_{solid} , the ordinary interference bands appear distorted, having a V-shaped notch. However, when $n_{\text{liq.}} = n_{\text{solid}}$, the bands have sharp straight edges, as if no crystal were in the beam at all. A measurement of n of the liquid gives n of the solid. The method, with modifications, is also applicable to anisotropic solids; for these substances, the specimens must be tested at various orientations with polarized light, as described by Wulff.⁵⁹

D. OTHER INTERFEROMETERS

The type of interferometer due to Jamin (1856) and modified by Zehnder and Mach (1891) was the first to be used for refractometric purposes and

¹⁶⁶ W. E. Williams, *Proc. Phys. Soc. London*, **44**, 45 (1932). Manufacturer, *Adam Hilger, Ltd.*, London.

still may be employed to advantage, particularly when the sample and the reference substance must be physically separated by a considerable distance from each other and from the other components of the apparatus, as in high-temperature work. Kessler¹⁶⁷ has described the various models and how to use them.

Rau and Roseveare¹⁶⁸ have described an easily constructed interferometer for liquids which can detect differences in n as small as 5×10^{-7} , using white light, and which has a range (0.002) exceeding that of the commercial instruments with the same sensitivity.

Lowry and Allsopp¹⁶⁹ have described an interferometer which photographically records the dispersion data for spectral lines in the range of 8000 to 2000 Å. An accuracy of 1×10^{-4} in n_λ is obtained. This instrument has been used for an extensive series of dispersion measurements of organic liquids within or near absorption bands, especially in the ultra-violet.

5. Photometric Methods

There are two principal ways in which the intensity of transmitted or reflected light may be related to refractive index:

(1) For unpolarized light incident at angle i with an intensity I_0 , the intensity I of the light reflected from the front surface is given by Fresnel's law:¹⁷⁰

$$I/I_0 = \frac{1}{2} \frac{\sin^2 (i - r)}{\sin^2 (i + r)} + \frac{1}{2} \frac{\tan^2 (i - r)}{\tan^2 (i + r)} \quad (68)$$

where r is the angle of refraction of the transmitted light. The meaning of this relationship in terms of refractive index is illustrated in the following special case.¹⁷¹ Assume that $i = 45^\circ$, and $n_0 - n$ is small, where n_0 , n are the refractive indices in the media of incident and transmitted rays, respectively ($n_0 \sin i = n \sin r$). Then equation (68) reduces to:

$$I/I_0 = \frac{1}{2} \frac{n_0 - n}{n} + \dots \quad (69)$$

(2) The intensity of transmitted light falls to zero when the angle of incidence equals the critical angle (see p. 1182). This is a special case of the general theory, expressed in part by equation (68).

The development of sensitive photoelectric cells has opened up the possibility of using the above principles and others to facilitate certain difficult

¹⁶⁷ H. Kessler, *Handbuch der Physik*. Vol. XVIII, Springer, Berlin, 1927, pp. 702-708.

¹⁶⁸ D. Rau and W. E. Roseveare, *Ind. Eng. Chem., Anal. Ed.*, **8**, 72 (1936).

¹⁶⁹ T. M. Lowry and C. B. Allsopp, *Proc. Roy. Soc. London*, **A133**, 26 (1931).

¹⁷⁰ F. A. Jenkins and H. E. White, *Fundamentals of Physical Optics*. McGraw-Hill New York, 1937, pp. 388-416.

¹⁷¹ F. W. Billmeyer, Jr., *J. Applied Phys.*, **18**, 431 (1947).

types of refractometric measurement. Invisible regions of the spectrum may be more easily explored, colored or translucent materials may be investigated. Perhaps the most important application of photocells will be to provide for automatic continuous recording of changes in refractive index.

Karrer and Orr¹⁷² have described several ways of making practical photoelectric refractometers. A very simple one is capable of 3rd decimal accuracy: a U-shaped glass rod is illuminated at one end and a photocell at the other end detects light which is transmitted by internal reflections. Upon immersing the curved part in a liquid, the intensity of the transmitted light is diminished by an amount which depends on the refractive indices of the liquid and of the glass. Photocell current *vs.* n is a smooth empirical curve. The electrical apparatus consists of a barrier layer photocell, a type K potentiometer and a galvanometer of 0.003 μ amp. per mm. sensitivity. Other arrangements permitted greater accuracy; a sensitivity in the 7th decimal has been claimed.

The Beckman spectrophotometer has been used by Lauer and Miller¹⁷³ as a source of essentially monochromatic radiation in a photoelectric refractometer designed to measure the critical angle between air and a transparent liquid. An air film, trapped between two quartz plates and immersed in the liquid, is rotated perpendicular to the incident radiation until the intensity of transmitted light falls off to zero, as indicated by a photomultiplier circuit. The dispersion in the ultraviolet down to 2000 Å. and into the infrared up to 1 μ can be determined within $\pm 2 \times 10^{-4}$.

Billmeyer has obtained the refractive index of solid films by changing the composition of a surrounding liquid until the intensity of reflected light reached a minimum; at this point the refractive index of the film was equal to that of the liquid, which could be measured (see Eq. 69).

Recording Refractometer.—An important recording photoelectric refractometer has been developed by Claesson¹⁷⁴ for the purpose of adsorption analysis. It includes a device for simultaneously recording the weight of liquid having passed through the sample cell. The complete instrument, capable of an accuracy of 1×10^{-5} , is available commercially.¹⁷⁵ It is applicable to a variety of problems involving continuous observations, such as distillations, titrations, extractions, and reaction kinetics. With quartz optics the apparatus would be suitable for ultraviolet work; with rock salt optics and thermopiles the infrared region could be easily ex-

¹⁷² E. Karrer and R. S. Orr, *J. Optical Soc. Am.*, **36**, 42 (1946).

¹⁷³ J. L. Lauer and P. H. Miller, Jr., *J. Optical Soc. Am.*, **37**, 664 (1947).

¹⁷⁴ S. Claesson, in *The Svedberg, 1884-1944*. A. Tiselius and K. O. Pedersen, eds., Almqvist and Wiksells, Uppsala, 1944.

¹⁷⁵ L. K. B. *Produkter Fabriks, AB*, Alvik, Stockholm.

plored. Using improved photocells which have since become available, the accuracy could probably be increased to 1×10^{-6} .

The optical principle is similar to other differential displacement methods (see p. 1218) and the photocells merely offer a convenient means of detecting the displacement. The refracted beam is split so that part of its radiation falls on one photocell and the remainder falls on an opposed cell. Any motion of the beam results in a relative change in illumination which can be translated into a galvanometer deflection and recorded on photographic paper.

6. Measurement of Gases and Vapors

The refractive indices of a gas are almost invariably determined with an interferometer. The principle of the method is the same as that discussed on pp. 1220–1226. The instruments described on pp. 1226–1233 can be used if they are provided with chambers of about 50–100 cm. length. Watson and Ramaswamy¹⁷⁶ describe the technique of handling gases and easily condensable vapors with the Rayleigh–Haber–Löwe interferometer. Cuthbertson and Cuthbertson¹⁷⁷ and Lowery¹⁷⁸ describe the use of the Jamin interferometer. Wüst and Reindel⁶³ and others⁴² adapted a modified Zehnder interferometer to the measurements of gases at high temperatures.

In order to obtain the molar refraction of a gas it is necessary to correct the experimental values of n obtained at arbitrary temperatures and pressures to n^* (see, e. g., ref. 63), of an ideal gas, usually at 0° C. and 760 mm. Since n^* differs little from 1, the Lorentz–Lorenz expression assumes the simplified form $R = \frac{2}{3}(n^* - 1)$ 22415, which is sufficiently accurate for most purposes. The methods of making the correction from n to n^* are given in references 63 and 176–178. The considerable deviation between reported n^* values for even such gases as methane ($R_p = 6.58^{179}$ and 6.50^{180}), ethane (11.47^{146} and 11.38^{181}), and propane (16.40^{179} and 16.09^{176}) may be due partly to improper correction to the ideal gas state and partly to impurities. Watson and Ramaswamy¹⁷⁶ and Weiss¹⁸² point out that measurements of easily condensable vapors must be carried out at low pressures to avoid large errors due to adsorption on the chamber windows.

¹⁷⁶ H. E. Watson and K. L. Ramaswamy, *Proc. Indian Acad. Sci.*, **A4**, 675 (1936); *Proc. Roy. Soc. London*, **A156**, 144 (1938).

¹⁷⁷ C. Cuthbertson and M. Cuthbertson, *Proc. Roy. Soc. London*, **A135**, 40 (1932); *ibid.*, **A97**, 152 (1920); *ibid.*, **A84**, 13 (1910).

¹⁷⁸ H. Lowery, *Proc. Phys. Soc. London*, **40**, 23 (1927); *Proc. Roy. Soc. London*, **A133**, 188 (1931). H. Huxley and H. Lowery, *ibid.*, **A182**, 207–216 (1943).

¹⁷⁹ S. Friberg, *Z. Physik*, **41**, 378 (1927).

¹⁸⁰ T. Larsen, *Z. Physik*, **111**, 394 (1938).

¹⁸¹ H. Lowery, *Proc. Phys. Soc. London*, **39**, 421 (1927).

¹⁸² M. Weiss, *Ann. Physik*, **20**, 557 (1934).

7. Techniques for Small Amounts

Alber and Bryant¹⁸³ and Wilson¹⁸⁴ have reviewed the micromethods applicable to organic liquids. Valuable suggestions are contained in an early paper by Wright.¹⁸⁵ Jelley describes these and other micromethods in chapter XV (see especially pages 938–942). The accuracy attained by the various techniques on amounts of sample ranging from 0.02 to 0.0001 ml. varies between ± 0.005 and ± 0.0005 in n .

A. ADAPTATION OF STANDARD INSTRUMENTS

Liquids.—Using an Abbe refractometer, one can reduce the amount of sample to about 0.01 ml. by distributing the liquid on lens paper, which is then clamped between the two prisms¹⁸³ (see also p. 939). An auxiliary glass plate (n_p), preferably roughened on one surface, serves to confine a thin layer of liquid ($n_l < n_p$) on the prism of the Pulfrich refractometer, converting it to an Abbe-type instrument. The usual precautions for obtaining grazing incidence must be observed (see p. 1212).

The microscope equipped with a graduated fine adjustment, but without unusual auxiliary apparatus, is capable of being used as a refractometer. An accuracy of ± 0.005 is possible on about 0.005 ml. of sample (4-mm. thick layer); even less sample is required if a smaller accuracy is permissible. All of the various techniques¹⁸⁶ are based on the depth-of-focus (image displacement) principle of de Chaulnes.

Solids.—The method of Le Blanc (see page 1200) with the Pulfrich refractometer requires only a few milligrams of the powdered isotropic substance. The accurate interferometric method of Wulff (see page 1232) can be employed with crystal fragments as small as 0.5 mm. in cross section (*i. e.*, $\cong 0.2$ mg.). The microscope (chapter XV) is often used.

B. IMAGE-DISPLACEMENT METHODS FOR LIQUIDS

Jelley,¹⁸⁷ Edwards and Otto,¹⁸⁸ Nichols,¹⁸⁹ and Alber and Bryant¹⁸³ have described simple refractometers which allow the rapid measurement

¹⁸³ H. K. Alber and J. T. Bryant, *Ind. Eng. Chem., Anal. Ed.*, **12**, 305 (1940).

¹⁸⁴ C. L. Wilson, *Analyst*, **71**, 117 (1946).

¹⁸⁵ F. E. Wright, *J. Washington Acad. Sci.*, **4**, 269 (1914).

¹⁸⁶ H. Kessler, in *Handbuch der Physik*. Vol. XVIII, Springer, Berlin, 1927, p. 716. See also E. M. Chamot and C. W. Mason, *Handbook of Chemical Microscopy*, 2nd ed., Vol. I, Wiley, New York, 1938, pp. 358–384.

¹⁸⁷ E. E. Jelley, *J. Roy. Microscop. Soc.*, **54**, 234 (1934); *Kodak Research Labs. Abr. Sci. Pub.*, **17**, 18 (1935).

¹⁸⁸ A. E. Edwards and C. E. Otto, *Ind. Eng. Chem., Anal. Ed.*, **10**, 225 (1938).

¹⁸⁹ L. Nichols, *Natl. Paint Bull.*, **1**, 12 (1937).

¹⁹⁰ Jelley–Fisher Refractometer, *Fisher Scientific Co.*, Pittsburgh, Pa.

¹⁹¹ Nichols Refractometer, *Arthur H. Thomas Co.*, Philadelphia, Pa.

of small samples. Two of these are available commercially,^{190, 191} but satisfactory models can be easily constructed in the laboratory. In all these instruments, the liquid sample is made into a minute 45° prism by placing a drop of it between an appropriate set of glass surfaces. If an object (*e. g.*, an illuminated line or slit) is viewed through such a liquid prism, its apparent position is shifted from the true position by an amount which can be observed on a scale mounted behind the prism or within an eyepiece lens system. The scale readings can be converted into the corresponding values of n by use of equation (1) or, better, by a calibration with standard liquids.

*The Jelley-Fisher refractometer*¹⁹⁰ (Fig. 38, Ch. XV) has a scale which gives values of n directly to ± 0.002 in the range of 1.30 to 1.90. Samples as small as 1×10^{-4} ml. have been measured to ± 0.001 with a modification of this instrument.¹⁸⁷ Liquids of high dispersion cause the slit image to be fringed with color, due to the white light source. Frediani¹⁹² has proposed that the width of this colored band be used as a semiquantitative measure of the dispersion. Following a suggestion given in Jelley's original paper,¹⁸⁷ Frediani¹⁹² has described a method for applying the instrument to high temperature measurements (up to 200°C.). A controllable electric heating device is attached to the cell which shapes the liquid prism. In this way, a variety of substances which are ordinarily solid may be measured in molten state. The m. p. (to $\pm 3^\circ$) can be conveniently obtained at the same time.

*The Nichols refractometer*¹⁹¹ consists of two prismatic cells mounted on a microscope slide, one of which is used in the ranges of $n = 1.30$ to 1.40 and 1.65 to 2.0 , the other between 1.40 and 1.65 . The cells require about 0.005 ml. of sample, 60% of which can be recovered. A microscope having an eyepiece scale is used to measure the separation of two displaced images of an illuminated reference line scratched on the slide. A calibration curve is obtained from measurements on a set of standard liquids. The calibration is simplified by the fact that the distance of separation of the images is a linear function of the refractive index. The usual accuracy of ± 0.001 can be increased to ± 0.0005 by using monochromatic light, and by controlling the temperature to about $\pm 0.5^\circ$. A cylindrical water jacket surrounding each cell may be cemented to the slide, or a hot stage may be used for temperature control.

Schlieren.—Applicable to extremely small particles of liquid or solid which can be suspended in a medium of adjustable refractive index is the method of Exner.¹⁹³ It resembles the Schlieren technique discussed on page 1219. The object casts a shadow on an image unless the two media are closely matched. Differences as small as 1×10^{-4} can be detected. In biological work the method has been used to characterize living cells.

¹⁹² H. A. Frediani, *Ind. Eng. Chem., Anal. Ed.*, **14**, 439 (1942).

¹⁹³ H. Kessler, in *Handbuch der Physik*. Vol. XVIII, Springer, Berlin, 1927, p. 717.

8. Guide for Selection of Methods

Table X is designed to facilitate the selection of instruments available commercially for various types of problems. Some of the instruments in table X can be modified, as described in the text, to allow a greater accuracy than indicated in the table; *e. g.*, a commercial interferometer can be adapted to an accuracy of 1×10^{-8} , compared with the usual 5×10^{-7} .

TABLE X
RECOMMENDED INSTRUMENTS FOR VARIOUS PROBLEMS

Required conditions	Recommended instrument	Usual accuracy, n_D or (Δn)	Page
A. PURE LIQUIDS			
High accuracy in n_λ	Precision Abbe	3×10^{-5}	1209
Rapidity, convenience	Abbe	2×10^{-4}	1203
B. SOLUTIONS OR PURE LIQUIDS			
Highest accuracy in n_λ^a	Spectrometer	1×10^{-8}	1217
Volatile component and/or hygroscopic	Pulfrich	1×10^{-4}	1183
Accuracy in dispersion $\Delta n = n_{\lambda_1} - n_{\lambda_2}$	Pulfrich	2×10^{-5}	1202
Detection of small concentration changes, $\Delta n = n - n_0$	Pulfrich	2×10^{-5}	1194
Routine analysis of solutions	Interferometer	5×10^{-7}	1220
Precision comparison, $\Delta n < 0.05$	Dipping	7×10^{-5}	1214
Rapidity, convenience, no temperature control	Pulfrich	3×10^{-5}	1194
Small amounts (10^{-2} to 10^{-4} cc.)	Jelley-Fisher	2×10^{-3}	1237
	{ Abbe	2×10^{-4}	1236
	{ Image displacement	5×10^{-4}	1236
Low temperatures	Pulfrich	1×10^{-4}	1183
Temperatures up to 100°C .	Pulfrich	1×10^{-4}	1190
Temperatures up to 200°C .	Jelley-Fisher	2×10^{-3}	1237
Continuous recording	Claesson photoelectric	1×10^{-5}	1234
C. SOLIDS			
Large, homogenous specimen, high accuracy in n_λ	Precision Abbe	3×10^{-5}	1209
Accuracy in dispersion, $\Delta n = n_{\lambda_1} - n_{\lambda_2}$, large homogenous specimen	Pulfrich	2×10^{-5}	1199
Opaque solids	Abbe	2×10^{-4}	1206
Semisolids	Abbe	2×10^{-4}	1206
Isotropic powders, $n < 1.9$	Pulfrich, Le Blanc	2×10^{-4}	1200
Small anisotropic crystals ($n < 1.9$)	{ Immersion, microscope	1×10^{-3}	930
	{ Immersion, interferometer	3×10^{-5}	1232
Shaped, polished specimen, highest accuracy in n_λ^a	Spectrometer	1×10^{-8}	1217

^a Any value of n .

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SPECTROSCOPY AND SPECTROPHOTOMETRY

W. WEST, *Eastman Kodak Company*

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I. INTRODUCTION

This chapter is essentially limited to a description of the general instrumental methods in spectroscopy which seem applicable to the solution of some of the specific problems of organic chemistry. The spectral region of interest is a wide one, extending from the infrared to the far ultraviolet. The growing recognition of the power of infrared investigations for some purposes for which visible and ultraviolet spectra are less fruitful justifies

a description of infrared spectroscopy. Practically all varieties of spectroscopic techniques are adapted to problems in organic chemistry, but greatest emphasis is laid on absorption spectroscopy. The investigation of feeble emission spectra such as Raman, fluorescence, phosphorescence, and chemiluminescence spectra can also yield results of interest to the organic chemist, and a section on Raman spectroscopy describes the general spectroscopic practice in such experiments.

II. THE SPECTRUM

1. Regions of Spectrum

It is well known that visible light is comprised within a region of wave lengths between about 7000 Å. in the red and about 4000 Å. in the violet. *Ultraviolet light* has shorter wave lengths than violet. It is conveniently regarded as subdivided into two spectral regions, that of wave length varying from about 4000 to 2000 Å., readily transmitted by quartz and air, which will be termed simply the ultraviolet region, and that of wave length shorter than about 2000 Å., the far ultraviolet, only a short region of which is transmitted by quartz and in which oxygen absorbs more or less strongly. Evacuated spectrographs must therefore be used in this region. The region from 2000 to 1200 Å. was first investigated extensively by V. Schumann by means of fluorite optics and is consequently often called the Schumann region. Similarly, the name of Theodore Lyman, who opened up the region of wave lengths shorter than those transmitted by fluorite by means of vacuum grating spectrographs, is associated with the spectrum below 1200 Å. The vacuum ultraviolet merges into the soft x-ray region. The *infra-red* spectrum comprises radiation of wave length just longer than that of the extreme red to about 5 mm., bordering on the high-frequency "radio" waves.

The unit of wave length commonly used for visible and ultraviolet light is the angstrom unit, Å., equal to 1×10^{-8} cm. The millimicron, $m\mu$, equal to 10 Å., is also in use. The unit used most often in the infrared is the micron, denoted by μ , equal to 10^{-3} mm., or 10,000 Å. The part of the infrared spectrum of greatest chemical interest extends from about 30 μ to the visible red. The frequency, $\bar{\nu}$, of a train of monochromatic radiation is related to the wave length by the relation $\bar{\nu} = c/\lambda$, where c is the velocity of light, 2.99776×10^{10} cm. per sec., and λ is the wave length. When c is in cm. per sec. and λ is in cm., $\bar{\nu}$ is in sec.^{-1} ; the unit of frequency so expressed is the fresnel.

More often used than the frequency in the description of spectra is the quantity $\nu = 1/\lambda$, the wave number of the monochromatic train, that is, the

number of waves per unit of length. The unit of wave number usually employed is the reciprocal centimeter, cm^{-1} . For many theoretical purposes, frequencies and wave numbers, and particularly differences in these quantities, are more significant than wave length and differences in wave length. As for the choice of frequencies or wave numbers, the vital consideration appears to be that the great bulk of the extant data in spectroscopy is in wave numbers, and, while there is in Kayser's *Tabelle der Schwingungszahlen*¹ a ready means of converting measured wave lengths to wave numbers (corrected to values *in vacuo*), there is no such table of frequencies.

2. Nature of Spectra

Spectra can be classified as emission and absorption spectra. An emission spectrum is obtained by the spectroscopic analysis of the light emitted by a luminous source, such as a flame, electric arc, or spark, or in phosphorescence, fluorescence, or scattering, and consists of a set of bright lines or bands against a dark background. An absorption spectrum is produced by placing an absorbing medium between a luminous source and the spectroscope. The medium abstracts certain wave lengths from the spectrum of the source, and the appearance of the spectrum is of a set of dark lines or bands against a bright background.

A continuous spectrum is one in which all wave lengths are represented, in emission or absorption as the case may be, over a relatively large spectral region. In a discontinuous or discrete spectrum the emission or absorption is confined to groups of wave lengths separated by regions of no emission or absorption.

The fundamental process that occurs in the emission or absorption of light by an individual atom or molecule is its transition from a state of one definite energy to another state of lower or higher energy. If the molecule, under the influence of radiation, undergoes a change from a state of energy E_1 to a higher state of energy E_2 it will absorb monochromatic light of frequency $\bar{\nu}$ such that $E_2 - E_1 = h\bar{\nu}$, where h is the universal Planck's constant, having the value, if the energies are measured in ergs and the frequencies in sec^{-1} , of 6.624×10^{-27} erg sec. Not all energy changes can be effected by interaction between the molecule and the radiation field; only if the change in state involves a change in the value of some permanent or induced electric moment in the molecule can the transition be effected by the absorption or emission of radiation, *e. g.*, mere changes in translational kinetic energy of the molecule are without effect on the radiation field. To a first approximation, the internal energy of a molecule can be regarded as composed of additive contributions from the electronic mo-

¹ Hirzel, Leipzig, 1925. For corrections of this table, see *Phys. Rev.*, **48**, 98 (1935).

tions within the molecule, the vibrational motions of the constituent atoms and the rotational motion of the molecule as a whole. As is well known, the energies of these various contributions are "quantized," *i. e.*, in a given state the isolated molecule may possess one out of, usually, a discrete set of values. Transitions between such states, if they are brought about by the action of light, therefore produce a spectrum consisting of discrete lines, spaced more or less openly as the distances between consecutive energy levels in the quantized sets are greater or less. In *atoms*, in which only changes in electronic energy are effected by light, the energy levels are relatively far apart, and the spectrum consists of a relatively open-structured set of lines, of which a familiar example is the visible spectrum of mercury vapor as emitted by a mercury lamp. Even complex atomic spectra, as that of iron in the arc, can easily be recognized, by means of instruments of quite moderate resolution, to be composed of fine discrete lines.

The quantized rotational and vibrational motions of molecules add spectral complications not present in atoms. Like atoms, molecules possess sets of electronic levels whose members may differ by large amounts of energy, transitions between which involve high frequencies in the visible and ultraviolet. But in any electronic state a molecule may exist in a number of vibrational states, separated by relatively small energy differences, and in any vibrational state many rotational states are possible, separated by still smaller energy differences. Thus there exists for molecules with a permanent electric moment, a *pure rotational spectrum*, in the far infrared, caused by changes in the rotational states in the lowest vibrational and electronic states; while, in the near infrared, combined vibrational and rotational changes without change in the electronic energy produce the *rotation-vibrational spectra*. When the quantum absorbed by a molecule is large enough to produce a change in electronic energy, a great many rotational and vibrational changes are also possible, so that what would appear in the spectrum of an assembly of atoms as a single sharp line becomes in the spectrum of an assembly of molecules a very complicated pattern of lines caused by the superposition of the vibrational and rotational changes on the electronic change. Under low dispersion, the spectrum of a molecular gas associated with a single electronic transition assumes the form of a number of bands, each one associated with a definite vibrational change. On further resolution the bands may be found to be composed of a large number of lines, each associated with a definite change in rotational state. The characteristic banded appearance of molecular spectra at moderate dispersion has given rise to their description as *band spectra*.

Not all spectra can be resolved into fine lines at high resolution. True *continuous spectra* exist, indicating that at least one of the set of levels in-

volved in the transition is not discretely quantized, but composed of energy levels infinitesimally close to each other. The existence of a continuous

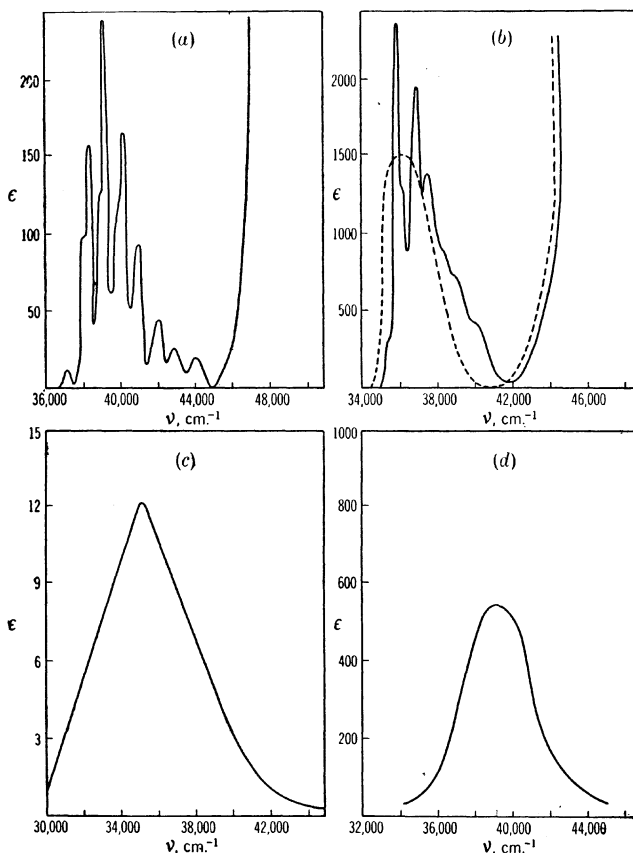


Fig. 1.—Types of absorption in the ultraviolet.

a, Benzene in hexane; *b*, phenol in (solid line) hexane and (broken line) alcohol; *c*, acetaldehyde in hexane; *d*, ethyl iodide in hexane.

spectrum in dilute gas indicates some kind of dissociation of the emitter—dissociation into a positive ion and an electron, or also, in the case of molecules, sometimes into uncharged fragments. In liquids and solutions,

however, mutual interaction between the absorbing molecules, or interaction between absorbing solute and solvent molecules, broadens the energy levels, with the results that discrete rotational structure in the spectra almost invariably disappears and sometimes the vibrational structure is also lost. The appearance of regions of continuous spectra in the condensed phase therefore does not necessarily imply ionization or dissociation, and in general will not do so if the gas has a discrete spectrum in the same region.

Examples of common types of absorption spectra in solution are shown in figure 1, illustrating the intensity of absorption, as measured by the

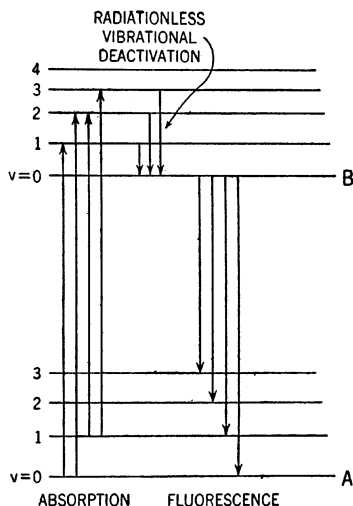


Fig. 2.—Relationship between absorption and fluorescence.

molar extinction coefficient, ϵ (see page 1297), as a function of wave number. The absorption spectrum of benzene (Fig. 1a) is one in which the vibrational structure is well marked in solution, a characteristic of conjugated ring hydrocarbons and some of their derivatives, though not confined to these compounds. In figure 1b, the absorption spectrum of phenol in hexane is seen to resemble that of benzene, with some displacement to lower frequencies; in alcohol, the banded appearance is lost, and the absorption region seems continuous. Nevertheless, the appearance of the vibrational bands in hexane (not to mention the application of chemical common sense) indicates the error of interpreting the continuous spectrum of phenol in alcohol as a dissociative continuum, although it is also obvi-

ous that there are rather strong interactions between phenol and alcohol molecules. Acetaldehyde in hexane (Fig. 1c) is a doubtful case: the spectrum is without vibrational structure, while in the gas both banded and continuous spectra appear in this region. Ethyl iodide (Fig. 1d) shows a continuum in this region both in the gas and all solvents, and the absorption appears to be accompanied by dissociation. From these examples it will be seen that comparison of spectra in the gas and in a variety of solvents, as well as consideration of chemical and photochemical evidence, is required before any very safe conclusion can be drawn as to the process associated with a continuous spectrum in solution.

Fluorescence represents the return of an optically excited molecule to its lowest electronic state by the emission of radiation. Whereas absorption at room temperatures occurs with greatest intensity in transitions from the lowest vibrational state of the lowest electronic state, *A* (Fig. 2), to a number of vibrational states in the upper electronic state, *B*, the return in fluorescence in solution is from the lowest vibrational state of the upper electronic state (vibrational deactivation in the higher electronic state taking place very rapidly by collision) to vibrationally excited and nonexcited states of the lower electronic state. The quanta emitted in fluorescence are therefore smaller than some of those absorbed, that is, the wave length of some of the emitted light is longer than that absorbed (Stokes' law).

A brief discussion of the nature of Raman and infrared spectra is given on pages 1371 and 1332.

3. Conversion Factors

In the theoretical consideration of spectral data, it is often necessary to convert from wave lengths or frequencies to energies and vice versa. The fundamental equation relating the frequency and wave length of monochromatic radiation absorbed to the energy gained by a gram molecule of absorbing substance is $E = N h \nu = N h c / \lambda$ where N is Avogadro's number. The energy in N quanta of frequency ν is the einstein unit of that radiation. Energy is often expressed in electron volts, the unit of which is the energy acquired by an electron in falling through a potential drop of one volt. A molecule which has absorbed radiation of frequency ν gains V electron volts of energy, where $eV = h\nu$, e being the electronic charge.

The following conversion formulas are easily deduced from the above equations:

$$\text{Calories per gram molecule} = 2.8579 \times 10^8 / \lambda (\text{\AA.}) = 2.8579 \nu (\text{cm.}^{-1})$$

$$\text{Electron volts} = 12,395 / \lambda (\text{\AA.}) = 1.2395 \times 10^{-4} \nu (\text{cm.}^{-1})$$

$$\text{Calories per gram molecule} = 23,055 \times \text{electron volts}$$

4. Summary

In figure 3 are summarized some important data relevant to spectroscopy, including the divisions of the spectrum, the molecular processes associated with each region, and the type of spectra to which they give rise, the transparency of materials useful for optics and windows, the methods of record-

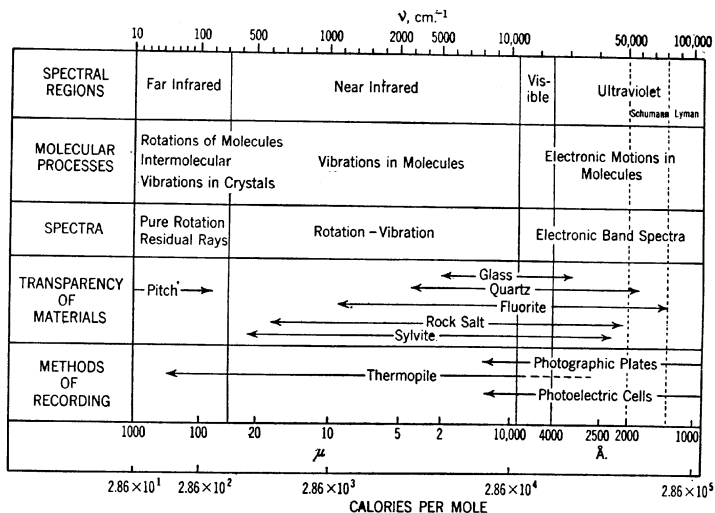


Fig. 3.—Summary of spectroscopy.

ing, and the value of the einstein at various frequencies throughout the spectrum.

III. SPECTROMETRY IN THE VISIBLE AND ULTRAVIOLET

1. Instruments and Their Characteristics

Examination of a spectrum involves an evaluation of position, intensity, and, sometimes, state of polarization, of the spectral features. That part of the operation concerned with determination of the wave length of absorption or emission bands forms the subject matter of *spectrometry*—measurement of intensities as a function of wave length is the object of spectrophotometry.

The instruments concerned in spectrometry are spectroscopes, spectrometers, spectrographs, and monochromators. As its name implies, the spectroscope is used for a visual examination of a spectrum; most useful spec-

troscopes have some means of permitting wave length estimation and are, strictly speaking, visual spectrometers. For permanence of the record and for precision in wave length determination, a photograph of the spectrum is much preferable to a visual examination, and regions of the spectrum capable of affecting the photographic plate, *i. e.*, from about 1.2μ in the infrared to the far ultraviolet are nearly always so recorded by means of spectrographs.

A monochromator is an instrument for isolating narrow wave length regions from a source of heterogeneous light. Except in the few spectral regions permitting isolation of different wave lengths by taking advantage of the difference in focal length of lenses for different wave lengths (the focal-isolation method of the infrared and short ultraviolet regions), monochromators, like spectroscopes, disperse the heterogeneous radiation by prisms or gratings, and the construction is essentially the same, except that in the monochromator a narrow band of the dispersed radiation is isolated by a narrow exit slit. Monochromators are used along with some kind of radiometer in most nonphotographic methods of spectrophotometry.

A. PRISM SPECTROGRAPHS

General Principles.—The essential parts of a prism spectrograph are shown in figure 4. The light to be analyzed enters the instrument through a slit, *S*, whose function is to provide a narrow aperture from which light

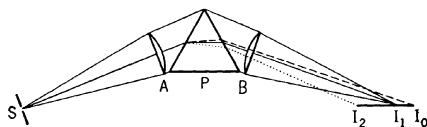


Fig. 4.—The prism spectrograph.

can reach every part of the collimating lens, *A*. The slit is placed at the principal focus of the collimating lens for light of a wave length approximately intermediate between the extremes to be registered by the instrument, so that for this light, from the points of the slit, a set of parallel beams is thrown on prism *P*. The prism is placed in the position of minimum deviation for this wave length, the rays then passing parallel to the base of a 60° prism and emerging symmetrically with respect to the incident beam. The condition of minimum deviation of a parallel beam is that of best definition of the refracted image. The parallel diffracted beam of wave length λ_1 , originating from a point in the slit, is focused by the camera lens, *B*, at point I_1 , which then represents an image of the point of the slit formed by light of wave length λ_1 . The images of the various points of the slit formed by light of wave length λ_1 combine to form the corresponding spectral line.

If the light entering the slit also contains a wave length λ_2 shorter than λ_1 , and if the collimating lens, as is usual in quartz spectrographs, is not achromatic, the beam of the shorter wave length will leave it slightly converging (the focal length of a nonachromatic lens diminishing with decreasing wave length). As a result of the greater refractive index of the prism for the shorter wave length, the refracted beam of wave length λ_2 is deviated more than that of λ_1 . The image of the slit formed by light of wave length λ_2 will be I_2 .

Similarly, if the light entering the slit contains a wave length λ_0 longer than λ_1 , its image will be formed at I_0 on the other side of I . If these three wave lengths comprise all the light entering the slit, a photographic plate placed in the image plane would record three separate images of the slit at I_0 , I_1 , and I_2 , which would constitute the spectrum of the light under examination. The length of the spectral line imaged by the camera lens is equal to that of the illuminated portion of the slit multiplied by the ratio of the focal length of the camera lens to that of the collimator. It will be noted that, strictly, only one image can be formed under the condition of best definition, but as small departures from minimum deviation produce little deterioration of the image, well-designed instruments have good definition over most of their range, except perhaps near the extreme edges of the plate, in instruments of large wave-length range. The collimating lens of most quartz spectrographs is a single lens uncorrected for chromatic aberration; the camera lens is usually composite, but also not achromatized. The focal plane is therefore markedly tilted with respect to the axis of the instrument. In modern instruments, the lenses are so figured that the images produced by different wave lengths lie very nearly in a plane, so that flat photographic plates may be used; in older instruments the images are brought to a focus on a curved surface and films or thin plates capable of assuming the curvature must be used.

The image formed by a prism-lens spectroscope adjusted for the incidence of parallel light on the prism set at minimum deviation, is approximately *stigmatic*, i. e., every point on the slit is brought to a focus at a corresponding point in the image. A dust particle in the slit therefore appears in a continuous spectrum as a dark line perpendicular to the length of the slit, and if adjacent portions of the slit are illuminated by different sources the respective spectra will appear adjacent to each other in the photographic

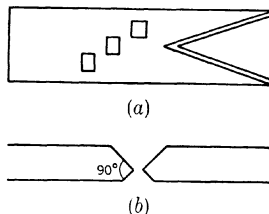


Fig. 5.—(a) Hartmann diaphragm.
(b) beveling of slits.

plate. The *Hartmann diaphragm* (Fig. 5) is a convenient means of exposing different portions of the slit length, and consists simply of a set of staggered holes in a metal plate mounted in grooves in front of the slit. By moving first one and then another hole over the slit, adjacent parts may be exposed to different spectra, *e. g.*, an unknown and a comparison spectrum can be photographed in juxtaposition without moving the plate holder.

Curvature of Spectral Lines.—The lines produced by a prism furnished with a straight slit are curved concave toward the short-wave side of the spectrum. Only the rays passing through the prism parallel to the base, *i. e.*, the rays from the center of the slit, traverse the path of minimum deviation; rays from the top and bottom of the slit are deviated more and cause the spectral lines to be curved. The use of an appropriately curved entrance slit eliminates the curvature (strictly only for one wave length) but this device is rarely used in spectrographs. In the use of monochromators, however, the impurity introduced into the isolated beam by curvature may be disturbing. These therefore are often provided with one slit curved for full compensation of the line curvature and the other straight, or with two curved each with half the curvature required for compensation.

The radius of curvature, ρ , of a spectral line formed in a prism instrument is:

$$\rho = \frac{n^2 f}{2(n^2 - 1)} \cot i$$

where n is the refractive index of the prism, f the focal length of the collimating lens, and i the angle of incidence of the light on the prism.²

Instrumental Details.—*Slits.* *Hartmann Diaphragm.*—Slits may be fixed, unilateral (*i. e.*, with one movable blade), or bilateral, with symmetrical motion of both blades.

Fixed slits are used only in the simplest instruments. The optimum slit width varies so much with the type of work to be undertaken that severe limitations are placed on the range of an instrument equipped with only a fixed slit. In some spectroscopic apparatus, a compromise is attempted between the simplicity of construction and permanence of adjustment of fixed slits, on the one hand, and a measure of adjustability, on the other, by furnishing the instrument with a series of slits accurately cut in a silvered layer deposited on a quartz plate. The plate is mounted in a slide in front of the collimator tube provided with stops to place any desired slit of the series in position.

The slit jaws should be in one plane, and should be provided with an adequately sensitive opening device with an attachment allowing the slit width to be read. Micrometer screws, sometimes graduated directly in

* H. Kayser, *Handbuch der Spectroscopie*. Vol. 1, Hirzel, Leipzig, 1900, p. 321.

microns of geometrical width, are available, though divisions of five or ten microns are more usual.

The unilateral slit, which combines high precision with relatively simple construction, suffers from the disadvantage that the center of the spectral lines at the photographic plate changes with the slit width. It is still used, however, in spectrographs of high quality.

Slit edges are beveled to a knife edge, as illustrated in figure 5b.

As no spectrograph is better than its slit, the greatest care should be used in the adjustment. It should not be closed tightly; some slits prevent this automatically. In cleaning from dust a sharpened soft wood splint should be carefully moved up and down several times between the jaws.

Modern slits are provided with a shutter, placed behind, so that when the shutter is closed the image of the light source can be set on the slit without affecting the plate.

The use of the Hartmann diaphragm in front of the slits of stigmatic instruments has already been referred to (page 1252). Another useful accessory, often combined with the Hartmann diaphragm, is a wedge-shaped diaphragm, whose position in front of the slit determines its effective height. The edges of the holes in a Hartmann diaphragm and the wedge edges of the wedge diaphragm should be beveled with the sharp edge toward the slit in order to prevent loss of definition of the top and bottom of the spectral lines by light reflected from the edges.

The construction of a good adjustable slit requires mechanical skill and proper facilities. Nevertheless, if high resolution is not demanded, a quite serviceable slit can be improvised by sticking good safety-razor blades by means of a soft wax onto a flat brass plate with a hole for the entrance of the light. Adjustment for width and parallelism can be made under a low-power microscope.

Materials for Prisms and Lenses.—For the optics of spectrographs in the visible and ultraviolet, practically only two classes of substance will be of interest to the organic chemist: glass and crystalline quartz. Flint glass prisms are transparent from about $2\ \mu$ in the near infrared to the near ultraviolet, the denser flints having an appreciably yellow color. Thin sheets of glass are transparent to about $3200\ \text{\AA}$., but fall off rapidly in transmission below this wave length.

Crystalline quartz is the material *par excellence* for prisms for the ultraviolet down to about $1850\ \text{\AA}$. Its dispersion in the visible is less than that of glass, but rapidly increases in the ultraviolet. But whereas well-annealed glass is an isotropic substance, the crystalline nature of quartz presents certain anisotropies which have no counterpart in glass. As a positive uniaxial crystal, quartz exhibits double refraction; *i. e.*, rays passing through quartz, except in one direction called the optic axis, are divided into

two, so that, in general, double images of a single object are produced. The effect of this double refraction in quartz prisms and lenses is eliminated by cutting them so that the light passes parallel to the optic axis.

In addition, the optical activity of quartz is associated with double refraction depending on the different velocities of propagation in the material of right- and left-hand circularly polarized light. This second cause of image doubling is not eliminated by passing the light along the optic axis, but is by building up a 60° prism of two 30° prisms of right- and left-hand quartz (*the Cornu prism*) and by pairing the lenses similarly. Since the circular double refraction is reversed if the beam is returned on itself through the medium, the effect is automatically eliminated in instruments of the Littrow type (page 1256).

Pure fluorite is transparent through much of the infrared, all the visible, and the ultraviolet into the far ultraviolet. Its use in the near ultraviolet is not common because of its expense and low dispersion, and although quartz-fluorite achromats can be made, most commercial spectrographs are not provided with them. Achromatization by quartz-lithium fluoride doublets has also been effected.

Fused quartz is not as transparent as the crystalline material toward the shorter wave lengths and is rarely satisfactory optically for prisms and lenses within the spectrograph, though condensing lenses of fused quartz in front of the slit are useful.

Not as transparent as quartz, but sometimes useful for windows, etc., down to about 2500 \AA. , are the Corex glasses of *Corning Glass Works*.

Types of Prism Spectrometers and Spectrographs.—This section will be limited to a brief description of some instruments likely to be of most use to the organic chemist.

Constant-Deviation Spectrometer.—A visual examination of the position of absorption bands is of little more than qualitative importance, and the spectrometer as such will not often be used. In conjunction, however, with a photometer, the spectrometer is of great use in visual spectrophotometers; moreover, they can be supplied with cameras to form useful spectrographs of moderate dispersion for the visible region.

By far the most convenient spectrometer is the constant-deviation instrument permitting direct reading of wave lengths, examples of which are now made by most of the manufacturers of prism spectroscopic equipment. The prism, illustrated in figure 6, can be regarded as built up of two 30° prisms which perform the separation of the rays and a totally reflecting 90° prism. If an incident ray is deviated through 90° by this prism, it passes through at minimum deviation, irrespective of the refractive index. If, therefore, the collimator and telescope tubes of a spectroscope are rigidly set at 90° to one another, the particular wave length seen at the cross hair

has passed through the prism at minimum deviation, and as the prism is rotated about an axis perpendicular to the plane of figure 6, different wave lengths are seen in the telescope at the position of minimum deviation. The motion of the prism is effected by a screw which pushes against a projecting arm of the prism table. Connected to the screw is a drum permitting the direct reading of the wave length of the line under observation with an accuracy of a few angstrom units.

Adjustment of wave-length scale can conveniently be made by means of a mercury lamp (p. 1280). The green line, 5461 \AA ., is brought on the cross

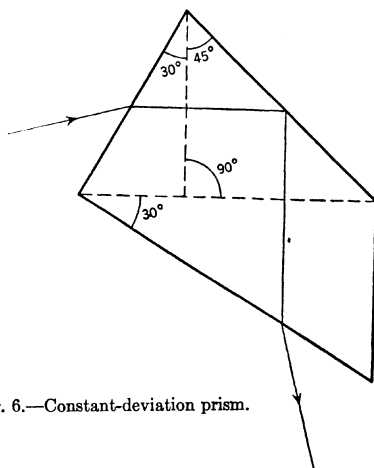


Fig. 6.—Constant-deviation prism.

hairs by manipulating the drum and screw; if the reading does not correspond to the known wave length, the screw is set so that the known wave length is indicated by the index, and the prism is slightly moved until coincidence of the line and cross hairs is achieved; or the line may be set on the cross hairs and the drum moved independently by loosening its connection with the screw until the known wave length coincides with the index. The calibration of the remainder of the scale is checked against the other known lines of the source. The wave lengths of the strong visible mercury lines are contained in table III, page 1281.

The telescope of constant-deviation spectrometers is customarily replaceable by a camera, usually of about one-meter focal length, giving photographs of the visible spectrum on a plate 3.25×4.25 inches. The length of spectrum from about 7000 to 4000 \AA . furnished by these instruments is from about 30 to 60 mm., according to the dispersive power of the prism.

Small and Intermediate Quartz Spectrographs.—These normally employ the conventional disposition of slit, collimator lens, prism, camera lens, and camera of figure 4 (page 1250). The small commercial quartz spectrographs yield a spectrum from 8000 to 1850 Å. on a 4.25×3.25 inch plate. They are provided with a wave-length scale which can be photographed on the plate. These are exceedingly useful instruments within their limitations of dispersion and resolution, and much work in absorption spectroscopy has been carried out by means of them.

The medium spectrographs have the advantage of greater dispersion and resolution, and are adequate for the great bulk of absorption work in organic chemistry. In general, instruments of this type use lenses of about 60-cm. focal length and about 50-mm. diameter, and give a spectrum extending from the visible to 2000 or 1850 Å. on a 10-inch plate. In addition to the medium spectrograph, *Adam Hilger, Ltd.*, makes an intermediate

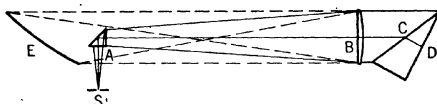


Fig. 7.—Littrow spectrograph (diagrammatic).

size, of focal length about 35 cm. for the D lines. The latest models use all-metal construction and, in some, curvature of the image field has been almost entirely banished, permitting the use of plates of ordinary thickness, and securing permanency of adjustment over the whole spectral region. Older designs have curved fields, and, in some cases, focusing adjustments have to be provided to allow the shorter wave end of the spectrum to be brought to best definition. Interchangeable glass-quartz optics are provided by some manufacturers.

Autocollimating Spectrographs.—Prism spectrographs of high dispersion employ lenses of focal length about 170 cm. or more, with prisms about 6 cm. high and 9 to 10 cm. in the length of the refracting face. Construction according to the design of figure 4 would yield an exceedingly cumbersome instrument; in practice, such instruments are made according to some modification of the autocollimating design of Littrow, in which the same lens acts both as collimating and camera lens. In figure 7, rays from slit *S* are reflected by a small quartz right-angle prism, *A*, along the axis of the instrument to collimating lens *B*. After passage through the 30° quartz prism, *C*, the rays are reflected back from front-surface mirror *D* (tin-mercury alloy or aluminum, not silver, which has a band of transparency about 3200 Å.), and the spectrum is focused by lens *B* at image surface *E*. Prism *A* is placed just below the axis and the spectrum is reflected so as just

not to be interfered with by *A*. Light which would be reflected directly on to the plate by the surface of lens *B* must be prevented from reaching the plate by the use of suitable baffles.

The spectrum is photographed on 10-inch plates, three plates being required to cover the range from about 8000 Å. to the ultraviolet limit. Instruments with the best quality quartz can transmit to about 1930 Å. or shorter. The prism and lens are mounted on a carriage which can be moved backward and forward for the photography of different regions, and the prism can be rotated. Both adjustments are made with the use of a scale and index. In the latest models of these instruments, all adjustments of the position of prism, lens, and plate holder are under the control of a single handle situated near the slit.

B. GRATING SPECTROGRAPHS

As is well known, a spectrum can be produced by a diffraction grating, that is, an assembly of very close parallel equidistant lines ruled on an appropriate surface. About 15,000 lines per inch is a common figure for visible and ultraviolet work; 30,000-lines-per-inch gratings are also used. The grating produces a series of spectra increasingly deviated from an undispersed "central image." The members of the series are called the first, second, . . . order spectra, counting from the central image. The dispersion in a given order spectrum increases with the closeness of the lines; and with a given grating the dispersion increases with the order. Gratings for the infrared regions are more coarsely ruled than those for the visible and ultraviolet.

Gratings directly ruled by the machine ("originals") are expensive, and normally are used when very great dispersion or resolving power is required, or, as reflection gratings, in regions where the opacity of prism materials precludes use of any other dispersing agent. Replicas, made by stripping a plastic coating like collodion from an original, are, however, relatively inexpensive and although usually lacking the performance of the originals in definition and resolution, form an excellent means of producing spectra when these demands are not too exalted. Especially is this true of the transmission replicas recently introduced for the visible by R. W. Wood, many of which appear to be not inferior in definition to their originals. In these gratings, the ruling is such that most of the diffracted light is thrown into a single order, with the result that the serious loss of light caused by the central image and higher orders of the ordinary grating is to a large extent eliminated.

Besides the plane grating, which, like the prism, requires collimating lenses and telescope or camera lenses, reflection gratings can be ruled on

concave spherical surfaces. The concave grating produces by itself a focused spectrum of a sharpness excelling that of prism-lens instruments, and without chromatic aberration. The image, however, is astigmatic unless auxiliary equipment is used, without which the use of Hartmann diaphragms and other devices for controlling the height of the spectrum is precluded.³ The plane grating produces a stigmatic image.

Only a brief description can be given here of the modes of mounting concave gratings. The classical *Rowland mounting* is now rarely used; the mounting most likely to be used in a chemical laboratory is that of *Eagle*. A characteristic of the concave grating is that, if the slit and grating are placed on a circle whose diameter is equal to the radius of curvature of the grating (the "Rowland circle"), the spectra are brought to a focus on this circle. Rowland's mounting does this automatically, also securing that the spectrum is observed normally to the grating. The spectrum thus produced is practically "normal," that is, equal distances of separation of spectral lines on the plate represent equal wave-length differences, but as the image is highly astigmatic, there is considerable waste of light. In Eagle's mounting, the slit, grating, and plate lie on the Rowland circle, but the grating is turned on its vertical axis until the diffracted beam nearly coincides with the incident beam. The focus is not automatically maintained as the grating is set for different wave lengths, and the grating is mounted on a carrier capable of motion to and fro along the line of sight. The plate holder must also be movable about a vertical axis.

The Eagle mounting is more economical of space than the Rowland, and the astigmatism is much reduced, with, therefore, less loss in intensity. The spectrum is not quite normal, but the deviations are not large, and linear interpolation between known wave lengths will be adequately accurate for most purposes of wave length determination.

Although the astigmatism of the concave grating used with other than normal incidence prevents the use of diaphragms, sectors, etc., immediately in front of the slit, there is a position in front of the slit at which a horizontal line will yield a sharp horizontal image at the plate. A narrow, totally reflecting prism introduced into the incident beam with its reflecting face along the horizontal focus line can therefore be used to reflect into the spectrograph a comparison spectrum, which will appear at the plate as a narrow band, sharply limited above and below, cutting across the unknown spectrum. Hartmann diaphragms and sectors can be set at the same place. This position is at the intersection of the line joining the slit and grating with the tangent of the Rowland circle at the position of the spectrum, and

³ See, for instance, H. T. Smyth, *J. Optical Soc. Am.*, **25**, 312 (1935). W. F. Meggers and K. Burns, *Bur. Standards Sci. Paper*, No. 441 (1922). R. F. Jarrel, *J. Optical Soc. Am.*, **32**, 666 (1942). J. W. Forrest and H. W. Straat, *ibid.*, **32**, 669 (1942).

rapidly increases in distance from the slit with increasing separation of slit and spectrum.

In some regions of the spectrum, the overlapping of orders of a grating spectrum presents some trouble, but not in first-order spectra of the ultraviolet and most of the visible. The second-order ultraviolet overlapping first-order visible can easily be removed by introduction into the light path, say before the slit, of a glass plate, opaque to the ultraviolet. Glass in the optical path in the spectroscope will serve this purpose. First-order spectra in the red and infrared fall in coincidence with second-order near ultraviolet and shorter visible, and appropriate filters have to be used in observations in these regions, in order to prevent confusion. The Corning Noviol filter, or the Wratten Filter 2A (*Eastman Kodak Co.*) is suitable.

Most gratings show the phenomena of "*ghosts*" to a greater or less degree, *i. e.*, lines appear in the spectrum at wave lengths at which the source has no line. The ghosts are best seen in line emission spectra. Rowland ghosts, due to periodic errors in the ruling screw, having the effect of superposing a low-dispersion grating on the ruled grating, are symmetrically placed with respect to and fairly close to their parent line, being, indeed, the spectra of the line produced by the subsidiary grating. They are easily recognized and usually cause no great trouble, at least in first-order spectra; their relative intensity increases with the order. Lyman ghosts, on the other hand, may occur at a position far from their parent line; strong Lyman ghosts make a grating difficult and uncertain to use.

Relative Merits of Grating and Prism Instruments.—Most spectroscopic work of interest to the organic chemist requires only moderate resolution and dispersion, so that the high resolving power of the grating in its higher orders is, in general, not an important point. A desirable feature of the grating is the approximate normality of its spectra, and, correspondingly, the crowding together of the longer waves in a prism spectrum is a disadvantage. The loss of light in the central image and unwanted orders, formerly a telling point against the grating, is greatly reduced in the type now available which concentrates most of the light in a given order. Overlapping of orders in the visible may have to be avoided by the use of filters.

In the organic laboratory, a prime requirement of a spectrograph would appear to be its suitability for spectrophotometric measurements. For these, as the discussion of spectrophotometry will show, a stigmatic image of the slit is desirable. The prism instrument holds a great and perhaps decisive advantage over a concave grating in this respect. It is true that stigmatic mounts of concave gratings are possible,³ but they tend to become cumbersome.

The plane grating with collimating and camera lenses gives a stigmati

image, and there seems no reason why, with the use of the echelette type of grating, concentrating the bulk of the dispersed light in a single order, an instrument that is useful for most purposes of the organic chemist cannot be made.

Large plane replicas seem to have a definite place in the field of high-aperture monochromators. Replicas four or five inches square are relatively inexpensive in comparison with prisms of the same aperture. There may be greater scattering of light by the grating material than by the prism, but the impurity thus introduced can be largely eliminated by using a double monochromator, *i. e.*, by sending the isolated beam transmitted by the first grating through a second. In expense, such a double monochromator would still be considerably less than a single-prism instrument of the same aperture. For use in the ultraviolet, the best construction would be reflection gratings and collimating and focusing mirrors (aluminized or platinized) rather than lenses.

C. FACTORS DETERMINING THE PERFORMANCE OF SPECTROGRAPHS

The performance of a well-made spectroscopic instrument is governed by three factors: linear dispersion, resolving power, and speed.

Linear Dispersion. Resolving Power.—The linear dispersion determines the length of the spectrum, that is, the distance on the photographic plate between two lines of given wave length separation, and is often expressed in angstrom units per millimeter of plate. It is to be carefully distinguished from resolution, which determines the minimum wave-length interval between two spectral lines that the instrument will show as distinct. For instance, it is possible, by displacing a prism from the position of minimum deviation, greatly to lengthen a spectrum, but at the cost of a more than proportional deterioration of the definition, with the result that resolution is lost. In a similar way, once the focal length of the camera lens is sufficiently great to eliminate the effect of graininess in the photographic image as a practical limit to resolution, further increase in focal length, although lengthening the spectrum, causes no increase in resolution.

The action of a prism in dispersing light depends on the fact that the velocity of propagation of shorter light waves in the material is less than that of longer waves, so that obliquely incident short waves are bent more than long waves. Since the refractive index of a material varies inversely as the velocity of propagation of light through it, the measure of the *dispersive power* of a material is the variation of its refractive index with wave length, $dn/d\lambda$. For many substances, the relation between n and λ can be expressed with considerable accuracy by the equation of Cauchy:

$$n = A + B/\lambda^2 \quad (1)$$

whence:

$$dn/d\lambda = -2B/\lambda^3 \quad (2)$$

i. e., the dispersion is inversely proportional to the cube of the wave length. As the wave length decreases, a given wave-length interval will, therefore, in a prism spectrograph for the visible and ultraviolet, be separated by a rapidly increasing distance on the photographic plate.

The phenomenon of a limited resolution not alone governed by dispersion originates, like the corresponding distinction in microscopy between magnification and resolution, in the fact that the image of a narrow slit with parallel sides is not geometrically similar to the slit, but consists of a pattern of maxima and minima of intensity of which the central maximum itself is spread out over a wider distance than corresponds to the image of geometrical optics. The pattern is the more widely spaced the narrower the beam incident on the prism. On the basis of an analysis depending on the criterion that two spectral lines of equal intensity, $d\lambda$ apart, will be perceptible by the eye as distinct when the central maximum of one falls on the minimum of the other, Lord Rayleigh obtained as the expression for *resolving power*, R , of a prism spectrometer:

$$R = \lambda/d\lambda = t \cdot dn/d\lambda \quad (3)$$

where λ is the mean wave length of the two lines, t the thickness of the prism base (the whole prism being filled with light), and $dn/d\lambda$ the dispersion of the prism material at wave length λ . This "theoretical resolving power" presupposes an infinitely narrow entrance slit, and, although deduced for visual observation, seems reasonably applicable for photographic recording when resolution is not determined by the graininess of the plate.

The resolving power of a grating for an infinitely narrow slit is simply equal to the product of the order of the spectrum and the total number of lines effective in producing the spectrum.

Slit Width and Resolution.—In practice, finite slit widths prevent the realization of the theoretical resolving power; moreover, for a given slit width in a given spectrograph, the resolving power depends on the mode of illumination. Schuster's analysis of the purity of spectra produced by finite slits, considered as sources of radiation, can be used as a guide to the slit widths to be used in practice when high resolution is required. The optimum slit width is about $4f\lambda/D$, where f is the focal length, D the effective horizontal diameter of the collimating lens, and λ the wave length. Narrower slits decrease the intensity of the spectral images of emission lines without greatly increasing the purity, while opening much above this value merely broadens emission lines, without increasing intensity, and seriously decreases purity. Continuous emission spectra increase in intensity as the slit is opened beyond $4f\lambda/D$ because of overlapping of the spectral elements

yielded by the infinitesimally close wave lengths, but at the expense of the resolution of structure.

The values of $4f\lambda/D$ for some common spectrographs are of some interest. Large quartz spectrographs have a focal length of about 160 cm. and a lens diameter of about 10 cm. At wave length $2500 \text{ \AA.} = 2.5 \times 10^5 \text{ cm.}$, $4f\lambda/D$ is 0.016 mm. For smaller instruments, $4f\lambda/D$ is about 0.01 mm. It is clear that, since no spectrograph is better than its slit, the utmost care must be taken to prevent damage to this essential.

The organic chemist, much of whose spectroscopic work will be on the broad bands exhibited by many organic substances in solution, will perhaps rarely be compelled to seek the maximum resolving power of his instrument. Nevertheless, the determination of resolution is probably the best test of the quality and adjustment of an instrument; and, when it is certain that the collimating lens is filled with light, an attainable resolution seriously lower than that indicated by the considerations mentioned is to be taken as evidence of faulty material, design, or adjustment. A suitable set of test lines is the triplet at 3100 (3100.671, 3100.309, 3099.971 \AA.) in the spectrum of the iron arc which should be easily resolved by a quartz spectrograph of medium size.

Slit Widths in Absorption Spectroscopy.—In absorption spectroscopy, the spectrum is produced by the removal of certain spectral regions by the absorbing substance, usually from a continuous background. So long as the actual width of the absorption band in wave-length units is considerably greater than the wave-length interval included by the image of the slit at the photographic plate, the absorption will be easily detectable; and, with due precautions, the intensity of the image with the absorbing medium in the light path as compared with that of the background at the same wave length without the absorbing substance in the path can be used to evaluate quantitatively the amount of absorption. If, however, the band covers a wave-length region narrower than that included by the image of the slit, the apparent intensity of absorption will be reduced from the true, and very narrow bands may completely escape detection. For instance, there is an appreciable concentration of hydroxyl molecules in heated water vapor, but the spectrum consists of the fine discrete rotational lines of an electronic band system, and only by the use of the highest resolving power is OH detectable by absorption against a continuous background.⁴ The effect of slit widths in quantitative absorption measurements is discussed on page 1303.

Cases of exceptionally narrow absorption bands sometimes arise in organic chemistry, as in the "cooperative absorption spectra" of mesomorphic

⁴ O. Oldenberg and F. F. Rieke, *J. Chem. Phys.*, **6**, 169 (1938).

forms of certain cyanine dyes,⁵ the absorption spectroscopy of which with instruments of moderate resolving power requires consideration of the requisitely narrow slit width.

With short-focus camera lenses, the resolution of the photographic plate may be the limiting factor in resolution. There is a limit (about 0.01 mm. in slow plates to 0.04 mm. in fast) to the distance between two lines that can be separately recorded by the plates likely to be used in spectroscopy; and to obtain the full resolving power of the optics of the spectrograph it is necessary to use a camera of focal length long enough to cause the linear separation between lines that can just be resolved to exceed the resolving power of the plate. This condition is fulfilled for medium-sized quartz instruments and the plates ordinarily used in absorption spectroscopy (see page 1382).

Speed of Spectrographs.—The time required to produce adequate density of the image in the photographic plate is of importance in the investigation of the spectra of feeble sources, as in studies of fluorescence and of the Raman effect. Although loss of light by reflection at the lens and prism surfaces and scattering within the optical materials affect the speed, these should be at a minimum in a well-designed and well-made instrument; and the relative apertures of the lenses allow rough comparisons of the speeds of different instruments. With a given slit width, prism, and collimating lens, two camera lenses of the same diameter, and therefore receiving the same amount of light, will produce images of area proportional to the square of their focal lengths; and, therefore, if losses by reflection and scattering are equal, the image intensities vary inversely as the square of the focal lengths. Some instruments, for example, those made by Zeiss, Steinheil, and Gaertner, and the Hilger constant-deviation glass spectrograph, are procurable with a variety of cameras, from those of long focal length to be used where dispersion is the important factor, to those of short focal length for work in which the feebleness of the light is the governing factor.

A rough guide to the relative speeds of different instruments is given by expressing the diameters of lenses as fractions of their focal lengths. Thus, a lens of focal length 160 cm. for the D line and diameter 8 cm. has an aperture for light of that wave length of $f/20$. Apart from losses by reflection and absorption (which may be serious in lenses made of numerous parts), the intensity of illumination of the image formed by the camera lens in a spectrograph is proportional to the square of this ratio. Medium-size quartz spectrographs, with a lens diameter of about 5 cm. and a focal length for the D line of 60 cm., have apertures for this wave length of about $/12$, while the large Littrow instruments with a lens diameter of, say, 7 cm.

⁵ E. E. Jelley, *Nature*, **138**, 1009 (1936). G. Scheibe, *Z. angew. Chem.*, **50**, 212 (1937). S. E. Sheppard, *Rev. Modern Phys.*, **14**, 303 (1942).

and focal length 170 cm. have apertures of about $f/24$. Other factors being equal, the medium instrument would furnish an image at the photographic plate four times more intense than that given by the larger instrument.

D. CHOICE OF INSTRUMENT

Spectrographs vary in size from those which spread the visible and ultraviolet spectrum over a considerable part of a circle of 21 feet radius, or more, to those which give a visible and near ultraviolet spectrum some millimeters long. Concave gratings are always used for the largest instruments. Their greatest use is in the investigation of detail in fine-line spectra; and although fruitful topics of study by the highest resolution are to be found in organic substances the use of these instruments is likely to remain within the province of the spectroscopist rather than of the organic chemist desirous of using the spectroscope as a tool in his chemical investigations.

The most common spectroscopic operation of the organic chemist is the determination of the absorption spectra of solutions, often including photometry. These spectra are nearly always rather broad continuous bands; even when well-defined vibrational band structure is present, as, say, in benzene, the bands are about 60 Å. wide, and the spectroscopic problem does not usually tax the resolution of the instrument. The large Littrow quartz instruments have a linear dispersion at 3000 Å. of approximately 5 Å. per mm., the medium-sized spectrographs, about 16 to 25 Å. per mm., and the small instruments, about 40 Å. per mm. In table I are included approximate values for the dispersion of the most commonly used quartz spectrographs.

TABLE I
DISPERSION OF QUARTZ SPECTROGRAPHS

Type	Focal length for D line, cm.	Dispersion, Å. per mm.		
		4000	3000	2000
"Small"	ca. 20	120	40	14
"Intermediate"	ca. 38	40	25	6
"Medium"	ca. 60	30	16	3
"Large" (Littrow)	ca. 170	13	5	1

For absorption work, it is best to avoid higher dispersion than is required. Broad bands of low intensity which are obvious at a glance in a spectrum at moderate dispersion may escape careful visual scrutiny of the spectrogram at very high dispersion. The "medium" and "intermediate" instruments are of greatest general use, and even the "small" quartz spectrographs have proved of great use in the study of the spectroscopy of organic compounds.

E. TESTING THE INSTRUMENT

In examining the performance of an instrument after delivery from the manufacturer, a test of resolution, as by photographing the iron arc and seeking the resolution of the 3100 Å. triplet (p. 1262) is probably the best procedure. Details are on p. 1291 in connection with the adjustment of focus. In the latest designs, the only adjustments the user should make are those of slit width and focus, and if, with adequately narrow slits and the aperture of the collimator filled with light, definition is unsatisfactory, there is nothing the user can do except consult with the manufacturer. In the matter of testing a spectrograph as delivered by the maker, reference should be made to the article on specifications and testing of spectrochemical apparatus by Sawyer and Vincent.⁶ To quote from this paper: "Test plates submitted by the manufacturer should not be relied upon unless they have been taken under carefully specified conditions. They may otherwise have been taken with weak exposures and high contrast plates—a condition which . . . suppresses the effects of stray light, of faint line components, and of poor line definition. High contrast plates are useful for practical work but should not be used for tests of quality."

2. Practice of Absorption Spectrometry

The essential features of an absorption spectrum are: the wave length or the frequency of the light absorbed; the intensity of absorption at different wave lengths; and, in oriented structures like crystals or films, the relation between intensity of absorption and the direction of vibration of the incident light with reference to axes determined by the orientation of the structure. In the absence of electrical or magnetic fields, the absorption of gases, liquids, and solutions which are optically inactive in the ordinary sense of organic chemistry does not depend on the state of polarization of the incident light, and a complete description of the spectrum is given by the spectrophotometric curve.

Mere visual inspection or a photograph of a spectrum will not permit the accurate determination even of the wave length of maximum absorption, when, as is usually true, the distribution of energy in the source is not independent of wave length. Nevertheless, it is usually convenient to begin the investigation of a new substance by exploratory work with the spectrograph alone; and there are many occasions in which the qualitative information provided by a photograph is adequate for the purpose in hand. Moreover, it is advisable to preface spectrophotometry with a purely spectroscopic examination at high resolution (narrow slits). As will be discussed at greater length in the section on spectrophotometry (page 1303), the

⁶ R. A. Sawyer and H. B. Vincent, *J. Optical Soc. Am.*, **31**, 47 (1941).

reliability of photometric measurements depends on the slit width used and the structural details of the spectrum, and these must be known before an intelligent choice of slit width in the photometer can be made.

To obtain an absorption spectrum, light, preferably from a continuous source, is focused on the slit of the spectrograph so as to cause the aperture of the collimator lens to be filled with light, passing in its path through the substance contained in a suitable tube with plane parallel windows. Care is taken that the absorption tube, lenses, and source are placed on the collimator axis.

A. LIGHT SOURCES FOR VISIBLE AND ULTRAVIOLET RADIATION

In this section a survey is made of the principal types of light source used in visible and ultraviolet spectroscopy. Similar sources are used in other branches of physico-organic chemistry, for instance, in polarimetry,

TABLE II
SUMMARY OF SPECTROSCOPIC SOURCES

Continuous Background for Absorption Spectroscopy

Visible: Tungsten filament lamps with straight coil or ribbon filaments, *e. g.*, projection lamps, phototube excitor lamps, automobile lamps. For moderate constancy use batteries (automobile lamps) or a. c. with voltage-regulating transformers.

For uniform illumination of an extended field: Ribbon filament lamp
Pointolite (page 1271), or zirconium arc (page 1271).

Ultraviolet: Hydrogen lamp, high- or low-voltage type (pages 1272, 1275).

Tungsten filament with quartz window (feeble below 3000 Å.)
Underwater spark.

Tungsten spark (not continuous) (page 1278).

Vacuum ultraviolet: Lyman source (page 1277).

Infrared: Nernst glower, Globar (page 1357).

Small Intense Continuous Sources for Visible and Near Ultraviolet

Plain carbon arc: About 170 candles per mm.² (page 1271).

"Concentrated arc" (zirconium lamp): About 100 candles per mm.² (page 1271).

Pointolite: About 10 candles per mm.² (page 1271).

Calibration Sources

Iron arc (Pfund): Secondary and tertiary standards for visible and ultraviolet (page 1287).

Mercury arc: H-4 for visible and near ultraviolet; small quartz arcs for ultraviolet (p. 1280).

Copper spark: For ultraviolet.

Numerous other metallic sparks and gas discharge tubes, *e. g.*, neon, helium.

Raman Source

Medium and low pressure quartz mercury arcs of high wattage (p. 1282).

Sources for Special Purposes

Sodium D lines: Sodium Labarc (page 1286).

Mercury 2537 Å. line: Mercury "resonance" arc, "germicidal" lamp (page 1281).

(Chapter XXIII), refractometry (Chapter XX), photometry (Chapter XXII), and photochemistry, and it is hoped that the data may also be useful to workers in these fields. For convenience in discussing special features in the use of infrared emitters, these sources are described in the section on infrared spectroscopy.

Many useful sources, such as tungsten filament lamps and mercury lamps of various sorts, are manufactured primarily for lighting purposes, and are therefore specified in makers' catalogues in terms of their efficiency as illuminating agents. If the lamp is to be used as a visual source, this information is useful in aiding in the selection of an appropriate source for the problem in question. Often, however, as in spectroscopy and photochemistry, the matter of prime interest is the energy output of the source in quanta or ergs per second, and it is then necessary to consider the relation between the luminous and energetic output in order to judge the suitability of a source specified only in terms of luminous efficiency. The following paragraphs therefore contain a short discussion, first, of the units used in photometry of visible light as such, and second, of the relation between the visual and energetic outputs of sources in the visible part of the spectrum. It will be evident, of course, that specifications purely in luminous terms give no information on the behavior of the source in the infrared or ultraviolet regions.

Photometric Units.—Visible light is evaluated by stating the rate of flow of radiant energy in terms of its power to produce visual sensation. The rate of flow of light is called the *flux*, and the unit of luminous flux is the *lumen*. The *lumen* is defined as the luminous flux emitted in unit solid angle by a uniform point source of one candle, an arbitrary unit of luminous intensity defined by agreement of the national standardizing laboratories of France, Great Britain, and the United States. The total luminous flux in all directions from a point source of one candle is therefore 4π lumens. Thus, one lumen is the amount of luminous energy that crosses in one second a unit area of a sphere of unit radius at the center of which is a point source of one candle.

The *intensity of illumination* at a point in a surface is the number of lumens per unit area incident at the point. The illumination produced by the incidence of one lumen per square foot is the *foot-candle*: or, what is equivalent, the foot-candle is the illumination of the surface of a sphere of radius one foot at whose center is a point source of one candle. The total luminous flux falling on the spherical surface is 4π lumens, and the flux falling on one square foot of the surface is one lumen.

Similarly, the *meter-candle* or *lux* is equivalent to an illumination of one lumen per square meter, and the *phot* is the illumination equivalent to one lumen per square centimeter.

The efficiency of incandescent lamps is expressed in terms of the number of lumens emitted per watt of electrical energy consumed. For example, the total luminous output of a new standard 100-watt tungsten filament lamp is approxi-

mately 1630 lumens, and the efficiency is therefore 16.3 lumens per watt. The efficiency of modern tungsten lamps varies from about 8 lumens per watt for a 10-watt lamp to about 13 for a 60-watt lamp and about 18 for a 200-watt lamp.

Relation between Luminous and Radiant Output.—In attempting to correlate luminous output with radiant output in energy units, the difficulty arises that a given radiant flux does not produce the same sensation of brightness at all wave lengths. The variation of the relative luminous efficiency of radiation with wave length is expressed in a *relative visibility curve*, which varies with the intensity level of illumination and somewhat with the observer. A standard relative visibility curve has been adopted which closely approximates the observed average for daylight (see Chapter XXIII, Fig. 31, by Heller). The maximum in this curve, set at unity, is at 5550 Å., in the yellowish green region of the spectrum and measurement has shown that 1 watt of this radiation is equivalent to 621 lumens. At 5000 Å., at which the relative visibility is 0.323, 1 watt of radiation is equivalent to 200 lumens, and at 4000 Å. in the violet, to 0.25 lumen. Averaged over the visible spectrum, the visibility of solar radiation is about 103 lumens per watt, while the visible radiation of a tungsten filament at 3000° K. has a visibility of about 27 lumens per watt.

The performance of sources whose emission depends essentially on a high temperature, such as incandescent filament lamps, can be suitably described in terms of the radiation characteristics of the black body. The black body is one which absorbs totally all the radiation falling upon it; the total energy emitted and the spectral distribution of the emitted energy depend only on the temperature. The rate of flow of the total radiant energy from unit area of a black body is given by Stefan's law:

$$W = \sigma T^4 \quad (4)$$

where $\sigma = 5.735 \times 10^{-12}$ watt cm.⁻² degree⁻⁴. The spectrum emitted is continuous, with an energy maximum at a wave length inversely proportional to the absolute temperature, as expressed by Wien's displacement law:

$$\lambda_{\max} T = \text{constant} \quad (5)$$

the value of the constant being 0.2884 cm. degree. The spectral distribution of the black-body radiation is given by Planck's equation:

$$J_\lambda = \frac{c_1}{\lambda^5} (e^{c_2/\lambda T} - 1)^{-1} \quad (6)$$

in which J_λ is the energy radiated in watts per cm.² of surface of a plane black body, per unit solid angle normal to the surface, between wave lengths λ and $\lambda + 1$, the distribution being assumed constant over the interval, for a wave length interval of 1 cm. In this equation $c_1 = 1.177 \times 10^{-12}$ watt cm.² and $c_2 = 1.432$ cm. degree. The unit of wave length in these equations is the centimeter.

These equations show that as the temperature of a black body is raised the energetic output at all wave lengths is increased, and the wave length of maximum intensity is displaced to smaller values. At room temperature, λ_{\max} is at 9.6 μ , and

the visible contribution is vanishingly small; at 1000°K. at $2.88\ \mu$, at 2800°K. (the approximate operating temperature of the filament of a tungsten lamp) λ_{max} is still in the infrared, at $1.03\ \mu$, while at 6000°K. (the approximate surface temperature of the sun) λ_{max} is in the blue green, at $4800\ \text{\AA}$. Some 75% to 85% of the electrical energy input of standard tungsten filament lamps is radiated in the infrared mostly between the visible limit at about $7600\ \text{\AA}$. and $5\ \mu$.

The radiation from other continuous emitters than the black body can often be approximately color-matched against that of a black body at some temperature; the temperature at which a black body must be operated so that its spectral distribution of radiation is the same as that of another body is called the *color temperature* of the latter. The color temperature of a tungsten filament is somewhat higher than the actual temperature. The concept of color temperature is useful only in connection with the spectral distribution of radiation from incandescent bodies, and not in connection with the absolute amount of radiation emitted. The radiant flux per unit area of tungsten, as of all nonblack bodies, is lower at any wave length than that of a black body at the same temperature, the emissivity of tungsten at 2800°K. , for example, at $6650\ \text{\AA}$. being only 0.419 and at $4673\ \text{\AA}$, 0.456, of that of a black body at the same temperature.

Sources of Continuous Radiation in the Visible and Near-ultraviolet. Filament Lamps.—Tungsten filament lamps are convenient and easily procurable sources for the visible, near-ultraviolet, and near infrared. The infrared limit is determined by the transmission of the glass envelope, which begins to absorb appreciably at about $2\ \mu$, though there is some transmission up to about $5\ \mu$, while the ultraviolet limit is set by the absorption of the envelope, which becomes strong at about $3200\ \text{\AA}$. The wave length of maximum intensity in the spectrum of standard filament lamps is usually somewhat longer than $1\ \mu$.

For spectroscopic purposes lamps with relatively short vertical filaments are the most suitable. Projection lamps contain a series of closely coiled filaments in a vertical plane. By placing the plane of the filaments on the continuation of the collimator axis a linear image can be projected at the slit which is useful in cases in which great uniformity of illumination at all points of the slit is not essential. Lamps with coiled filaments are quite useful for visual observation of spectra and for some spectrophotometric purposes, as in substitution methods using photoelectric cells. Lamps with "prefocus" bases are useful with respect to easy replacement in an optical system. Projection lamps are available which consume from about 75 watts up; some have relatively narrow bulbs which require forced ventilation in operation, and others are mounted in wider bulbs which are kept adequately cool by natural ventilation. Photocell exciter and sound recorder lamps are small precision-made sources with a single straight coil made for use in sound reproduction systems and are convenient to install in optical systems. They require a source of lower voltage than the

normal house supply, for instance, a step-down transformer. A large variety of automobile lamps is also available with filament forms suitable for projection at a slit. Supplied from storage batteries of large ampere-hour capacity, they are convenient when, as in single-beam photometry, high constancy over short time intervals is required.

When a great constancy of illumination is required over the slit, or over extended fields, as in double-beam photometry, ribbon filament lamps with a filament considerably longer than the slit, or lamps of the Pointolite type, employing an intensely illuminated small sphere which can be projected to form a very uniform field, are usually recommended. The recently developed zirconium arc is well suited to this purpose. Ribbon filament lamps consume large currents at low voltages, conveniently supplied from a transformer of adequate power.

The radiant output of tungsten filament lamps can be considerably increased by applying a higher than normal voltage, at the expense of a rapidly diminishing life. Some relations concerning the variables of lamp behavior are summarized in the following equations:⁷

$$\begin{aligned} \frac{\text{lumens}_1}{\text{lumens}_2} &= \left(\frac{\text{volts}_1}{\text{volts}_2} \right)^{3.4} & \frac{(\text{lumens/watt})_1}{(\text{lumens/watt})_2} &= \left(\frac{\text{volts}_1}{\text{volts}_2} \right)^{1.9} \\ \frac{\text{color temperature}_1}{\text{color temperature}_2} &= \left(\frac{\text{volts}_1}{\text{volts}_2} \right)^{0.27} & \frac{\text{life}_1}{\text{life}_2} &= \left(\frac{\text{volts}_2}{\text{volts}_1} \right)^{13} \end{aligned}$$

An increase in voltage of 10% from the normal value is accompanied by an increase of 38% in luminous output, an increase of 19% in luminous efficiency, and an increase of 2.4% in color temperature, at the expense of a decrease in average life to 29% of the normal value. Very considerable gains in visible and near-infrared intensity can be realized by overloading an automobile lamp, as may be convenient, for instance, in the photography of near-infrared spectra at wave lengths at which the plate shows low sensitivity. It is also obvious from these equations that the normal fluctuations in line voltage cause considerable fluctuations in the radiant output of filament lamps, and if constancy is required some special provision must be made. Battery-run lamps are often an adequate solution to the problem of constancy; constant-voltage autotransformers are very useful if alternating current is used, and electronic devices whereby the lamp regulates itself through the intermediary of photoelectric currents generated by its light in a suitable photocell have been described for purposes requiring extreme constancy,⁸ such as a source furnishing an output of 0.1 lumen with an error of 9×10^{-6} lumen.

⁷ *General Electric Lamp Bulletin*, Lamp Department, General Electric Co., Nela Park, Ill.

⁸ B. Chance, *Electronics*, 13, 24 (Feb. 1940).

Enclosed Arc Lamps.—*Pointolite Lamp.*—A uniformly heated metallic sphere provides a source of uniform brightness, which by suitable optical projection can be used for the uniform illumination of extended fields. Lamps of the type of the Pointolite use this principle in the production of visible and near-ultraviolet continua. The anode is a tungsten sphere, varying from about 1.2 mm. in diameter in the smaller models to 6 mm. in the larger, and the cathode is a coiled tungsten filament, contained in a glass bulb with nitrogen at low pressure. The lamp uses a special starting device. After the arc is struck between the sphere and coil, a bimetallic strip moves the sphere to the working position, opposite a more robust part of the cathode. Some care is necessary in focusing the sphere on the slit of a spectrograph to avoid the appearance in the spectrum of nitrogen bands from the region between the electrodes. Lamps rated at 30, 100, 500, and 1000 candle power are available, the 100 candle power model having a brightness of 10 candles per mm.² Like all arcs, the Pointolite lamp has a "run away" current voltage characteristic, and a ballast resistance is included in the starting auxiliary.

Zirconium Lamp.—A recent development in enclosed arc lamps is the zirconium lamp, or "concentrated arc," the elements of which are enclosed in a glass bulb containing argon.⁹ The cathode is of specially prepared zirconium oxide, raised to its melting point by ionic bombardment. A circular spot on the cathode is the source, along with a cloud of excited zirconium vapor and argon extending a few thousandths of an inch from the surface. The cathode surface emits a continuous spectrum extending from the near ultraviolet to the infrared cutoff of glass, with a maximum near $1\ \mu$ (color temperature 2800°K.) while the vapor emits a continuum extending from the short ultraviolet to $5000\ \text{\AA.}$, overlaid by the lines of neutral and singly ionized zirconium and argon. The bright spot is from 0.075 mm. to several mm. in diameter, according to the size of the lamp, the small lamp being an approximation to a point source. The brightness of the small lamp* is approximately 100 candles per mm.².

Carbon Arc.—A simple arc circuit consists of two electrodes (made of carbon, iron, or another metal), in series with a resistance, connected to the direct-current mains, or to an alternating-current supply through a rectifier. (An inexpensive rectifier useful for the operation from a three-phase alternating-current supply of devices requiring direct current at 100–200 volts and several amperes is described by Welch and Blacet.¹⁰

* A photomicrographic lamp, consisting of a 40-watt zirconium arc with the necessary electrical and optical auxiliaries is manufactured by *Fish-Schurman Corp.*, 250 East 45 Street, New York.

⁹ W. D. Buckingham and C. R. Deibert, *J. Optical Soc. Am.*, **36**, 361 (1946).

¹⁰ G. Welch and F. E. Blacet, *J. Chem. Education*, **17**, 484 (1940).

For many laboratory purposes a current of 5–10 amp. is adequate, and the resistance should be chosen accordingly. The arc is started either by momentarily placing the two electrodes in contact (insulated handles) and then separating them, or by placing the electrodes in contact by means of a carbon or metal rod held in an insulating handle.

Carbon arcs have some uses as continuous sources, while metallic arcs are usually employed as line sources (see p. 1287). Better and more convenient sources, however, are usually available for most spectroscopic purposes. The characteristic features of the carbon arc are its high intensity, high color temperature, and relatively small size of the source; its chief disadvantages as a spectroscopic source are its tendency to wander in position and certain spectral peculiarities. In the "plain" carbon arc, the chief seat of emission is the positive crater, which emits a continuous spectrum, overlaid at about 5000 Å. by the Swan bands and in the violet and near ultraviolet by the cyanogen bands. The ultraviolet spectrum of the carbon arc is not very intense. The chief laboratory use of the carbon arc is therefore in applications requiring a concentrated source of high intensity in the visible, near ultraviolet, and infrared, in which variations of intensity can be tolerated. Excitation of fluorescence, phosphorescence, and in some cases, preparative photochemistry, microscopy, and photomicrography, are among such applications. The automatic arcs made by the manufacturers of photomicrographic equipment are probably the most convenient for most laboratory purposes.

Cored carbons may contain salts which increase the conductivity of the arc without contributing essentially to the spectral distribution (neutral cores), or salts such as those of strontium or calcium which contribute their own spectra. The latter are used in "flame arcs" which emit the bulk of their radiation from the "flame" between the electrodes.

The brightness of the crater of a plain carbon arc at full efficiency is about 170 candles per mm.².

Sources of Continuous Radiation for the Ultraviolet.—*High-Voltage Hydrogen Lamp.*—The best source of continuous radiation throughout the ultraviolet to about 1600 Å. is provided by an electric discharge through hydrogen at low pressure. Two types of hydrogen discharge are available, high voltage and low voltage, the latter using a heated oxide cathode. Both are represented in commercial lamps. A high-voltage tube, made entirely of quartz, and based on a design by Kistiakowski,¹¹ is supplied by the *Hanovia Chemical and Manufacturing Co.*, Newark, N. J., and an oxide-coated cathode tube by the *National Technical Laboratories*, South Pasadena, California.

¹¹ G. B. Kistiakowski, *Rev. Sci. Instruments*, **2**, 549 (1931).

The high-voltage tubes require a transformer delivering from 2200 to ca. 6600 v.; a 2- or 3-kw. transformer will enable the spectrum to be photographed at medium dispersion with 15 seconds or so exposure. Unless the design of the transformer is known to preclude the possibility of dangerous withdrawal of current when the lamp has started, a protective resistance or reactance in the primary should be introduced. A variable resistance limiting the maximum current in the primary to about 20 amp. will be adequate for most purposes.

High-intensity hydrogen lamps of the high-voltage type must be water cooled. In Kistiakowski's design, a water jacket is fused to the tube and, since the expensive tube can be destroyed if operated without cooling, it is

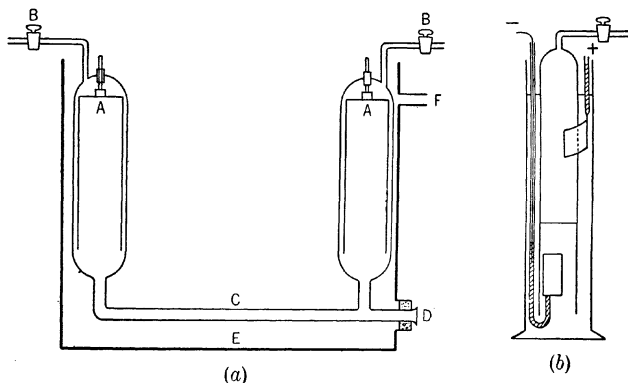


Fig. 8.—(a) Simple hydrogen tube. (b) Electrolytic generator.

advisable to mount it in conjunction with a switch, obtainable from electrical supply houses, which automatically cuts out the current if the water stream is not maintained. An hydraulic relay for this purpose is described by Bayley.¹²

Adequate hydrogen lamps for spectroscopy can be simply made from Pyrex glass, a quartz window being sealed on with Picein or other cement for the exit of the radiation. Figure 8 gives details of a tube which will provide a spectrum of sufficient intensity with quartz spectrographs of medium dispersion in exposures of under one minute when driven with about one kilowatt in the primary of a 3000-v. transformer. A represents cylindrical aluminum electrodes, about 6 inches long and 1.75 inches in diameter, attached to 2-mm. tungsten rods, and sealed in close to the glass

¹² D. S. Bayley, *Rev. Sci. Instruments*, **13**, 300 (1942).

wall. *B* represents stopcocks on the hydrogen entrance tube and on the pump connection. *C* is a Pyrex tube about 12 inches long and 0.5 inch in diameter. *D* is a quartz window sealed to the discharge tube with Picein, etc. *E* is a can for cooling water and *F* is the water exit.

Dry electrolytic hydrogen from a commercial tank or from a generator should be used to fill the tube. Figure 8*b* shows a simple electrolytic generator made from a liter measuring cylinder and an inner vessel. The electrolyte is dilute sulfuric acid; and platinum electrodes about 4 sq. in. in area should be used.

The hydrogen should be carefully dried, for instance by passing through concentrated sulfuric acid followed by phosphorus pentoxide. Otherwise the OH bands at about 3064 Å. and at shorter wave lengths will disturb the evenness of the spectrum.

To fill, the tube is attached to a vacuum pump and to the hydrogen train, is well baked out under vacuum to remove gas from the electrodes and glass walls, and is flushed out with hydrogen several times, with evacuations between fillings. A vigorous stream of water is made to circulate through the metal can, care having been taken, by proper disposition of the water exit tube, that the water level is below the tungsten seal. Gas is allowed to stream through the tube for some hours with the current passing, to allow for "cleanup" of the hydrogen. The optimum pressure for the continuous discharge is just below the highest pressure at which striations appear. It is convenient to observe the visible spectrum by means of a hand spectroscope before turning off the stopcocks. At first, the spectrum is likely to be impure, recognized by rather strong discrete bands in the green, blue, and violet, but after repeated flushing and passing of the discharge during the flow of hydrogen, the impurities disappear, and the hand spectroscope shows a great number of very fine lines in the yellow and green and the continuum in the blue, with the red and blue Balmer lines.

An electronic regulator for maintaining constant intensity of a high-voltage discharge tube has been described.^{12a} A pilot photoelectric cell in a compensated feed-back circuit controls the impedance in series with the primary of the high-tension transformer supplying the discharge tube so that the intensity changes less than 0.1% for a 10% change in line voltage.

Although the high-voltage hydrogen lamp is an excellent source for determining the structural details of an ultraviolet absorption spectrum, the extended nature of the light source, with consequent spacial nonuniformities in intensity, and also the low-frequency alternations in intensity (60 cycles per second or so) demand certain precautions in its use as a source

^{12a} W. L. Pondrom and W. W. Robertson, *Rev. Sci. Instruments*, **19**, 561 (1948).

for photographic absorption spectrophotometry by divided beam methods. These are discussed on page 1309.

Low-Voltage Hydrogen Arcs.—Arcs in hydrogen between an oxide-coated cathode and a molybdenum anode can be maintained at a relatively low voltage and have several advantages over the high-voltage type. An arc

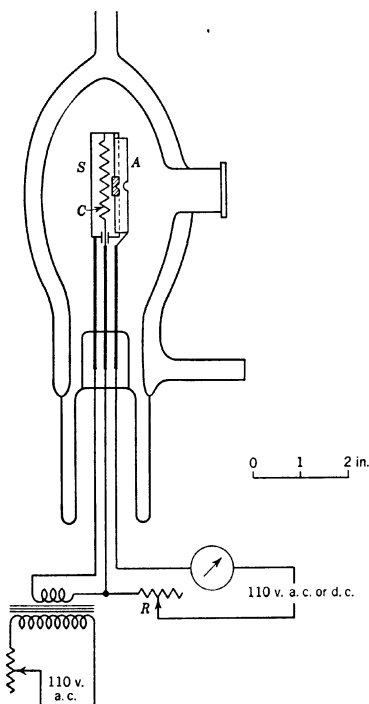


Fig. 9.—Low-voltage hydrogen lamp.

of this type which has been used in several laboratories is described by Allen and Franklin¹³ and is illustrated schematically in figure 9. The cathode consists of an oxide-coated filament, *C*, surrounded by a nickel cylinder, *S*. The filament is formed from a piece of nickel screen 0.25 inch wide and 5.5 inches long, made into a corrugated form coated with a mixture of barium and strontium oxides¹⁴ in equal proportions. The cathode

¹³ A. J. Allen and R. G. Franklin, *J. Optical Soc. Am.*, **29**, 453 (1939).

¹⁴ Obtainable from the *Callite Products Co.*, Union City, N. J.

cylinder, S , is $\frac{9}{16}$ inch in diameter and 2 inches long, made from nickel sheet 0.01 inch thick. One terminal of the filament is insulated from the shield by a small quartz tube, the other is connected to the cylinder. The orifice may be a hole 3 mm. in diameter in tungsten or molybdenum sheet, spot welded into a larger hole in the nickel; other sizes and shapes can be used. The anode, A , is a rectangular piece of molybdenum bent into the arc of a circle and provided with an orifice 5 mm. in diameter placed directly opposite the cathode orifice. The electrode assembly is mounted in a water-jacketed Pyrex bulb. A tubule carries a quartz window sealed on with glyptal lacquer.

The filament is heated to a red heat by a current of 10 or 12 amp. at 3.5 to 4 v. provided from a step-down transformer. The arc will run on both direct and alternating current, though direct current is more efficient. The arc voltage is about 40 v. and is regulated by the variable resistance, R , of about 100 ohms, capable of carrying 2 amp. The arc current should not exceed 1 amp. for continuous running or 1.5 amp. for short intervals. As soon as the arc is started, the filament current may be turned off; its life will be increased in this way.

Filling with hydrogen is performed as described for the high-voltage lamp. In degassing, the electrodes should not be brought to a red heat, as the nickel will become spongy and absorb gas on cooling. A very powerful source of this type, using 10 amp. at 60 v. in the arc, is described by Allen.¹⁵

The low-voltage arc readily permits the use of a small and uniform source, making it applicable to double-beam spectrophotometry without the precautions required with the high-voltage type. If the arc is furnished by direct current, there are, of course, no difficulties with stroboscopic effects.

According to observations of Bosch and Anderson¹⁶ the low-voltage arc of Allen and Franklin is highly satisfactory for absorption spectrophotometry with photoelectric cells using electrometer-tube amplification (page 1317). At an arc current of 1 amp., the intensity is sufficient to produce an easily measurable photoelectric response at moderate levels of amplification from a quartz-enclosed Cs-O photocell (G-M 1038A). The response-wave length curve for this cell to radiation transmitted by a monochromator from a low-voltage arc at 1 amp. is shown in figure 10. The amplifier sensitivity was 2.23×10^{-13} amp. input current per mm. deflection of the galvanometer.

Other sources than hydrogen lamps can be used as ultraviolet continua. Tungsten filaments enclosed in bulbs with quartz windows can be used:

¹⁵ A. J. Allen, *J. Optical Soc. Am.*, **31**, 268 (1941).

¹⁶ W. C. Bosch and E. W. Anderson, *Rev. Sci. Instruments*, **15**, 437 (1944).

they suffer from the disadvantage of the relatively low intensity in the further ultraviolet obtainable from any temperature radiator with a reasonably long life. Metallic sparks under water have also been used¹⁷ but this type of source seems to offer no advantages over the hydrogen tube.

*Continuous Source for Vacuum Spectroscopy*¹⁸.—The hydrogen spectrum produced as just described is not continuous at shorter than about 1600 Å., and a source described by Lyman is usually used in vacuum spectroscopy as a continuous background. A high-capacity condenser (0.5 to 8 microfarads have been used) is charged to a potential of 5000 to 15,000 v. by the

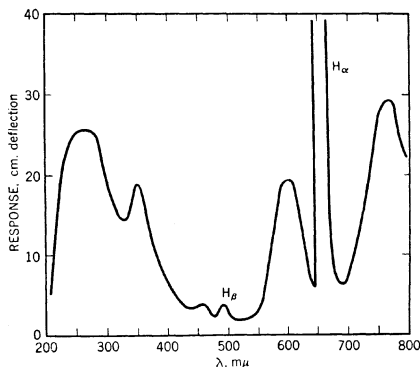


Fig. 10.—Response of quartz-enclosed Cs-O photocell to radiation from low-voltage hydrogen lamp (after Bosch and Anderson).¹⁶

rectified output of a high-tension transformer, of 1 or 2 k.v.a., and discharged two or three times per second through a narrow capillary of quartz or Pyrex, about 1 mm. in internal diameter and 20 to 30 mm. long. For a given quantity of electricity in the discharge, longer life and higher intensity are obtained from high capacity and relatively low voltage than vice versa.^{18a} Hydrogen or helium, purified by passage through charcoal or chabasite in liquid air, is passed continuously through the discharge tube at about 10^{-2} or 10^{-1} mm. of mercury. The discharge through the gas between electrodes at the two ends of the capillary is a damped oscillatory discharge of high current density, and is accompanied by the emission of an intense white light, whose spectrum is continuous from the visible to

¹⁷ See, for example, H. J. McNicholas, *Bur. Standards J. Research*, **1**, 939 (1928).

¹⁸ (a) G. Collins and W. C. Price, *Rev. Sci. Instruments*, **5**, 423 (1934). (b) A. B. F. Duncan, *Phys. Rev.*, **47**, 822 (1935). (c) F. W. Paul, *ibid.*, **52**, 926 (1937). (d) R. E. Worley, *Rev. Sci. Instruments*, **13**, 67 (1942).

800 Å. or shorter, overlaid, in the vacuum ultraviolet, by atomic emission lines of highly ionized Si, O, N, C, and by absorption lines of the neutral atoms.^{18c} Although sometimes inconvenient because of the break in the continuum, these atomic lines are useful for calibration.

Repeated flashes cause gradual widening of the capillary, accompanied by a decrease in the intensity of the continuum and increase in the intensity of the atomic lines, until eventually the capillary must be replaced after a relatively short life. The latter inconvenience has caused little use to be made of this type of source except in the vacuum ultraviolet. The intensity, however, is extremely high: according to Anderson,¹⁹ the brightness at 7000 Å. in a tube 4 mm. in internal diameter and 5 cm. long subject to the discharge of a condenser of 2 μf. charged to 30,000 v., corresponded to that of a black body at 50,000° K., and at 3500 Å. to that of a black body at 20,000° K. The source has been used occasionally in the visible and near ultraviolet in studies requiring high intensity and short duration. It has been used, for example, in the estimation of the life time of the CN radical by measuring the absorption spectrum of the near-ultraviolet cyanogen bands as a function of the time elapsing after excitation.²⁰

Reference perhaps should be made to the sources used in high-speed photography, which are capable of application to general studies demanding sources of high intensity for short times. A survey of such sources is made by Edgerton, Germeshausen, and Grier,²¹ along with detailed wiring diagrams for several specific purposes. These sources resemble the Lyman source in employing a condenser discharge through a gas-filled gap, though conditions are such that the inconveniently short life of the Lyman capillary is avoided. The sources are of two essential types: (1) those in which a relatively long period, of the order of 10 seconds, must elapse between flashes; and (2) stroboscopic light type apparatus yielding a high-frequency succession of flashes which can be synchronized with periodic phenomena. Commercial examples of both types are available. As an example of the possibilities inherent in condenser-discharge sources, mention may be made of a lamp exhibited by the *General Electric Co., Ltd.*, which, using a condenser of 150 μf. charged to 2000 v., delivered an output of 12,000 lumen-seconds, with a duration of about 150 microseconds and a maximum flux of about 40 million lumens.²²

Condensed Spark Sources.—For photographic spectrophotometry in the ultraviolet a condensed spark between tungsten steel electrodes or between one tungsten and one steel electrode is frequently used. The equipment required includes a suitably insulated holder for the electrodes, a small transformer about 0.25 kw., with a secondary open-circuit voltage

¹⁹ J. A. Anderson, *Astrophys. J.*, **72**, 137 (1930).

²⁰ J. U. White, *J. Chem. Phys.*, **8**, 79, 459 (1940).

²¹ H. E. Edgerton, J. K. Germeshausen, and H. E. Grier, *J. Applied Phys.*, **8**, 2 (1937).

²² *Photography*, **1**, 32 (1946).

of ca. 10,000–20,000 v., a condenser of about 0.005- μ f. capacity capable of withstanding the spark voltage and power, and some means of adjusting the primary current. A variable resistance or lamp bank can serve for this purpose; especially convenient are autotransformers of the "Variac" type (General Radio Co., Cambridge, Mass.). The Leyden jar (capacity about 0.003 μ f.) forms an inexpensive, adequate, but bulky condenser; much more compact condensers or "capacitances" are obtainable from the various manufacturers of radio apparatus. The end of the electrodes between which the spark is struck may be sharpened to a wedge, placed with the edge coaxial with the collimator.

The essentials of a spark circuit are shown in figure 11. *M* indicates a.c. house terminals, *R* the resistance or autotransformer, *T* the high-tension transformer, *C* the condenser, and *S* the spark gap. The connecting leads in the secondary circuit should be short and stout.

In emission spectroscopy, the spectral lines originating from air may be troublesome because of their overlapping the metallic lines. Their intensity can be reduced by introducing an inductance in the secondary circuit, in series with the spark. A suitable inductance can be improvised by winding about 50 turns of stout copper wire on a cardboard or Bakelite tube about 4 inches in diameter. Spectrograms of the source are made at various values of effective inductance by tapping at different positions on the coil, and the optimum conditions determined.

The tungsten spark is rich in lines, most of which are of about the same order of intensity and can serve as an approximation to a continuous source in absorption spectroscopy and spectrophotometry. Since the spark discharge is at high frequency, the source is free from trouble because of stroboscopic effects with the use of rotating sectors, and it has the merit of simplicity in assembly and in operation. On the other hand it is definitely not a continuous source, and fine details of structure may be difficult to find against the many-line spectrum. Low-voltage direct-current hydrogen arcs of the Munch and Allen types yield true continua and act as small sources suitable for spectrophotometry, although they are more difficult to assemble than the spark circuit.

In any case, a sparking apparatus is almost an essential piece of equipment in ultraviolet spectroscopy, for testing focus and for providing comparison spectra in wave-length determinations. The copper spark is rich in accurately measured lines in the shorter part of the ultraviolet, and aluminum, cadmium, zinc, and other metals are sometimes useful for calibration purposes.

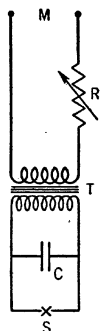


Fig. 11.—A spark circuit.

Mercury Arcs.—Mercury arcs are widely used as sources of discontinuous radiation in a wide variety of physical measurements. They are useful in spectroscopic calibration in the near-infrared, visible, and ultraviolet regions, the more intense varieties are used in the production of Raman, fluorescence, and phosphorescence spectra, and they are used as sources in polarimetry, refractometry, and interferometry and in photochemistry.

The older type of mercury arc had a cathode consisting of a pool of liquid mercury; the anode was either a similar pool or a tungsten electrode. The arc was started by tilting until contact was momentarily made between anode and cathode, after which the discharge continued with the emission of the mercury spectrum. As the arc heated, the pressure of mercury vapor increased, and the appearance of the discharge and the quality of the spectrum went through a series of changes until, if ventilation conditions were suitable, a steady state was reached, in which the arc operated with considerable constancy. Any change of conditions was however accompanied by a change in the output. The arc moreover usually had to be operated in a fixed orientation.

Arcs of this type are still common in laboratories, and can be obtained from the manufacturers. Most commercial mercury lamps now sold contain a small accurately measured amount of mercury which is wholly vaporized in operation; they start automatically, and, with some exceptions, can be operated in any position. They are much less sensitive to the ambient temperature than the pool type, provided, of course, that all the mercury remains as vapor.

Variation of Mercury Spectrum with Pressure.—Mercury lamps can be conveniently classified with respect to their radiation characteristics according to the pressure of the vapor under operating conditions. Table III lists the lines in the spectrum for arcs working at medium pressures (that is, of the order of one to several atmospheres, which is the value for most commercial lamps) and approximate values of the relative intensities of the stronger visible and ultraviolet lines, with respect to the group at about 3660 as 100. These intensity values cannot be used for quantitative, but only for orienting purposes.

The two lines at 2537 and 1849 Å., the so-called resonance lines, originate in transitions from excited levels to the normal electronic level, and correspondingly occur as absorption lines in cold mercury vapor. The lines are therefore readily reversed if any appreciable amount of cooler vapor surrounds the excited column in the lamp. The other lines involve transitions between excited electronic states.

Low-Pressure Mercury Lamps (Resonance Lamps).—At low pressures of mercury vapor, a quartz mercury arc emits chiefly the resonance lines,

2537 and 1849 Å., the latter strongly absorbed by fused quartz and somewhat by oxygen. Mercury resonance lamps are available commercially,²³ as straight tubes or coiled spirals or in other shapes made to order. The tube contains neon or another rare gas at a pressure of a few millimeters and is driven by a high-voltage transformer of the type used for illuminated signs. In operation, the tube becomes barely perceptibly warm; 90% of the radiation emitted is in the two resonance lines, of which that at 1849 Å. is considerably weakened by the envelope. The chief use of these tubes is as sources of radiation at 2537 Å.; they are, for instance, excellent

TABLE III
LINES OF THE MERCURY SPECTRUM FROM MEDIUM-PRESSURE ARCS

Region	λ	Relative intensity	Region	λ	Relative intensity
Infrared	3.6492 μ		Ultraviolet	3906.44	2.6
	1.3673			3663.27	
	1.3570			3662.88	100
	1.1287			3654.83	
	1.0140			3650.15	
Visible	6908 Å.			3341.47	
	6234.35			3131.84	60
	6123.46			3131.56	
	6072.64			3125.62	
	5790.66			3023.48	
Yellow	5769.60	88		3021.50	31
Green	5460.724	71		2967.28	
	4916.0			2803.5	
Blue	4358.343	58		2653.69	22
	4347.50			2652.07	
	4339.21			2576.31	
	4108.07			2536.520	28
Violet	4077.8			2534.78	
	4046.77	34		1942	
				1849	

sources for Raman excitation by that line. Several tubes can be laid alongside the vessel to be illuminated, or it may be surrounded by a spiral lamp. The low intensity of the other lines makes these lamps less useful than others for general spectroscopic purposes, although in applications in which especially narrow lines are desired, the feebleness of the radiation from resonance arcs might be tolerated.

The "germicidal lamps" commercially available emit most of their radiation at 2537 Å. They employ heated filament cathodes, run at low voltage, and have a tube made of an ultraviolet transmitting glass. These lamps have proved useful in ultraviolet photometry (page 1425).

Arcs which normally run at medium pressure, such as the pool type

²³ *Ultraviolet Technical Laboratories*, Los Angeles, Calif. *Hanovia Chemical and Manufacturing Co.*, Newark, N. J.

quartz mercury lamps, can be used as sources of wave length 2537 Å. if they are cooled, *e. g.*, by immersion in water. Reversal is still further prevented by deflecting the arc against the wall of the burner by means of a magnetic field: simple permanent magnets are adequate for this purpose.

Medium-Pressure Arcs.—Most mercury arcs operate at from about one to ten atmospheres. Included in this group are the quartz burners, both of the pool type and of the self-starting a.-c. type, as well as the glass-enclosed arcs like the H-type lamps of the *General Electric*, *Westinghouse*, *British Thomson Houston*, and other lamp companies.

Most mercury pool lamps operate only on d. c., although some can be used on a. c., working on the principle of the rectifier. It is very important for the life of the lamp that the polarity of d.-c. arcs be correct—the electrode nearer the constriction is the cathode. The transmission of quartz in the short ultraviolet gradually diminishes with the age of the lamp, especially if a dust layer is burnt in, and the lamp should be carefully freed from dust and grease by swabbing with alcohol before using. In using any quartz arc it is necessary to protect the eyes and skin from ultraviolet light; a painful conjunctivitis follows exposure of the eyes to this light, and erythema following exposure of the skin can be uncomfortable and, if frequently repeated, perhaps not without serious consequences to health. If possible the lamp should be mounted in a housing which prevents access of ultraviolet light to the room. Ultraviolet-absorbing glasses fitting closely to prevent access of radiation to the eye from the side should always be used in working about a quartz arc or any source of ultraviolet light; and if the skin is likely to be exposed protective measures should be taken. The small shower-bath hoods with transparent visors obtainable from department stores form not too inconvenient protection for the face, or the materials sold by druggists to prevent sunburn might be used.

Direct-current quartz arcs must be operated in series with a resistance large enough to absorb about 40% of the line voltage, and capable of carrying about 5 amp. The performance of the lamp is determined by the mercury pressure, the arc current, and the potential drop across the arc, any two of which determine the third. Just after starting, the pressure is low, the current high, and the arc voltage low. The glow, of relatively feeble luminosity, fills the tube. As continued operation heats the arc, the pressure rises, the potential drop increases, and the current falls, the arc becoming concentrated along the axis of the burner. The resonance line is then strongly reversed, and the other lines show some pressure broadening which is inconsequential for most purposes. In addition to the line spectrum, the medium-pressure arcs emit a relatively feeble continuous spectrum—the stronger the higher the pressure—overlaid by some diffuse bands, which can be disturbing in connection with Raman, phosphores-

cence, and fluorescence spectra excited by its means. For removal of this in the visible by means of filters see page 1373.

As previously stated, modern quartz ultraviolet lamps employ oxide-coated electrodes, a measured small amount of mercury, and argon at low pressure to initiate the discharge.^{24*} The tubes are about 0.75 inch in diameter and vary in length from 1 $\frac{3}{8}$ to 12 $\frac{1}{2}$ inches. For spectroscopic purposes such as calibration and testing of focus the smaller arcs are suitable. All these arcs start automatically and can be burned in any position. In normal operation, no liquid mercury is present, so that the final operating voltage is determined by the complete evaporation of the mercury. The output is thus less influenced by the ambient temperature than in the pool type, provided it is high enough to prevent condensation.

The lamp is driven by a transformer, incorporated in which is a reactor in series with the arc. The reactor limits the discharge current, stabilizes the arc, and minimizes flicker. The glow discharge of argon first appears when the lamp is started, which rapidly changes to the mercury discharge. The discharge becomes constricted as the droplets of mercury evaporate and, after a relatively short time, all the mercury is evaporated and the steady state is reached.

Table IVA-B gives data on lamps of this type (Uviarcs) made by the *General Electric Vapor Lamp Co.*, Hoboken, N. J., which are probably representative of the type in general.

For many laboratory purposes the small, glass-enclosed mercury quartz arcs made by several manufacturers for general lighting applications are very convenient and inexpensive sources of the visible and near-ultraviolet lines. In general characteristics, these lamps resemble the self-starting ultraviolet lamps just described, and they must be used with transformers of special design to furnish the starting and working potentials. In the 100-watt lamp (H-4 in the series of the General Electric Company's lamps of this type which may be taken as representative) the arc is struck in a quartz tube about 1 $\frac{3}{4}$ inches long and a few millimeters wide at an operating pressure of 8 atmospheres. The lamp can be used in any position. The glass outer bulb limits the lines transmitted to those of wave length longer than about 3200 Å.; a "sunlamp" using the same unit has an ultraviolet-transmitting glass transparent to about 2800 Å. An ultraviolet source can be improvised from the normal lamp by removing the outer envelope by means of a hot wire or other suitable cutter. As a source of the 3660 group nearly free from visible radiation, convenient for producing visible phosphorescence and fluorescence excited by this radiation, the 100-watt lamp is also supplied in an outer bulb of red-purple ultraviolet-

²⁴ L. B. Johnson and S. B. Webster, *Rev. Sci. Instruments*, **9**, 325 (1938).

* It should be remembered that a.c. Hg lamps are intermittent sources, the intensity nearly vanishing between peaks, and in any application careful consideration should be given to the advantages or disadvantages of this feature.

transmitting glass. These lamps start with a blue argon discharge, and are nearly at full intensity after about three minutes, although changes occur for about fifteen minutes after starting and to a slight extent later.²

TABLE IVA
DATA ON MEDIUM-PRESSURE QUARTZ UVIARCS

Arc watts.....	150	250	360	600	1200
Arc length, in.....	1.38	3	6	6	12.5
Internal diameter, in.	0.6	0.6	0.6	0.7	0.7
Arc volts.....	55	95	140	180	350
Amperes.....	3	3	3	4	4

TABLE IVB
RADIATION DATA FOR UVIARCS

Wave length, Å.	Microwatts/cm. ² at 1 meter				
	150 watt	250	360	600	1200
5791}	37.3	60.2	83	255	510
5789}					
5461	33.5	56.0	82	222	445
4358	28.5	45.5	72	192	385
4047	16.9	26.8	45	111	223
3654 group	49.6	78.5	122	327	654
3341	5.3	7.4	11.6	35	71
3129 group	32.6	50.8	85	203	406
3022 group	16.6	25.3	39.4	107	214
2967	8.8	13.5	20.9	56	113
2894	3.5	5.3	8.3	23	47
2804	7.0	10.9	15.7	42	85
2753	2.5	3.8	5.5	17	33.6
2700	3.0	4.4	7.6	20	40.2
2652	14.4	22.5	33.1	88	176
2537	15.5	27.8	47.4	103	207
2483	6.1	9.9	13.3	39	79.5

Among the laboratory uses of these small arcs are as sources for spectroscopic calibration and focus testing, in polarimetry, refractometry, and interferometry, in certain applications of colorimetry and photometry, and for excitation of fluorescence and phosphorescence. A larger source for the visible and near ultraviolet is the 400-watt lamp (H-1), with an inner glass bulb $7\frac{3}{4}$ inches long and about 1 inch in diameter, operating at slightly above 1 atmosphere. This arc can only be run vertically, but a similar lamp with a quartz inner tube and glass outer tube can be run horizontally. Good ventilation of the 400-watt lamp is required as overheating can cause melting of the inner tube.

Capillary Arcs.—Mercury discharges of very high intensity can be produced by restricting the arc within a short capillary tube, so that a large

² G. Wald, *J. Optical Soc. Am.*, **35**, 189 (1945).

amount of power is consumed in a small volume. Water-cooled and air-cooled lamps consuming about 1000 watts are made by several manufacturers. They attain a pressure of about 100 atmospheres in operation. It is recommended that water-cooled lamps of this type be used in conjunction with a pressure switch in the control circuit which will automatically cut off the power if the water flow is interrupted. These arcs emit a continuous spectrum of considerable intensity, as well as broad lines, and are unsuited for purposes in which purity of the mercury lines is desirable.

Quartz capillary mercury arcs of high intensity can be readily made in the laboratory. Figure 12 illustrates the simple design of Daniels and Heidt.²⁶ Quartz tubing 1 to 2 mm. in internal diameter and about 1 mm. in wall thickness is used. The bulbs *A* and *B*, which are blown in the gas-oxygen flame as thin as consistent with mechanical strength, form the limits of the arc, while *C* is an expansion chamber into which the mercury is forced when the arc is struck between *A* and *B*. The upper electrode (+), of tungsten or iron wire is sealed in with de Khotinsky cement before the lamp is filled, the end of the tubing being well filled with cement. Filling is accomplished by mounting the tube in a vessel with its open end under purified mercury, evacuating, and slowly admitting air until the mercury rises in contact with the upper electrode. The tube is inverted and the lower electrode sealed in with cement. The electrodes are connected to insulated wires and the exposed pieces covered with de Khotinsky cement. If used on a 110-d. c. line a resistance of 20 to 30 ohms (which can be improvised from a radiator heating element) should be placed in series with the lamp, higher resistances being required with lines of higher voltage.

Water cooling is essential in the operation of this lamp. A water vessel

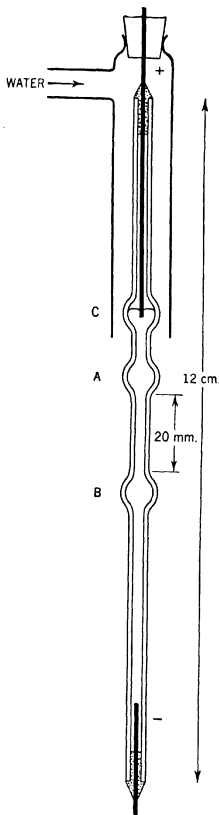


Fig. 12.—Daniels-Heidt capillary arc.

* F. Daniels and L. J. Heidt, *J. Am. Chem. Soc.*, **54**, 2381 (1932).

is supported as shown by a rubber stopper round the upper portion of the lamp. To start the lamp, after connection is made to the mains through the series resistance, the middle of the quartz tube is heated with a small Bunsen flame, or by means of a small heating coil above bulb *A*. As soon as the arc strikes a rapid stream of water is turned on. If the arc fails to start properly, it may be necessary to reduce the series resistance, or to decrease the level of the mercury, producing a greater volume for expansion, and consequently a lower pressure and lower resistance in the arc. Special precautions should be taken in the operation of this arc to avoid ultraviolet burns of the eyes and skin (page 1282).

Capillary arcs of this type operate at moderate pressure, usually not exceeding 5 atmospheres. The continuous background in the spectrum is therefore not unduly strong, and they are quite serviceable as line sources, for example, for some applications in Raman spectroscopy and in fluorescence experiments. Their life is short, say about 24 hours of operation when consuming about 1 kilowatt, though considerably longer at 300 watts, but they are inexpensively replaced. Other metals, such as Bi, Cd, Pb, Tl, and Zn, may be used in quartz capillary arcs.²⁷

Other Arc Line Sources.—*The sodium vapor lamp* is the modern equivalent of that ancient laboratory "monochromatic" source, the salt-fed Bunsen flame. The lamp consists of an inner bulb of resistant glass containing a small amount of sodium and neon at low pressure, placed within a double-walled vacuum vessel to maintain the proper temperature for operation. After preliminary heating of the cathodes, the circuit is broken, and the surge induced in the transformer windings starts a neon discharge. As the lamp warms, the sodium is gradually evaporated and after some time the characteristic yellow color assumes its full brilliance. Small laboratory models are available consuming about 28 watts, in addition to the larger sizes familiarly used in highway lighting.

The spectrum is mainly composed of the D lines, 5890 and 5896 Å.; the second members of the principal series at 3301 and 3303 Å. are also present, besides relatively feeble lines of the subordinate series. The neon spectrum is also emitted with appreciable intensity. If relatively pure D-line emission is desired, a filter should be used, *e. g.*, a layer of 7% potassium dichromate several cm. thick.

*A cesium vapor lamp*²⁸ has been recently developed, 50-, 100-, and 500-watt models being available. Most of the radiation is in the near infrared resonance doublet, 8521 and 8943 Å. Visible radiation can be excluded by means of a Wratten filter No. 87. The source should be useful for spec-

²⁷ R. M. Hoffman and F. Daniels, *J. Am. Chem. Soc.*, **54**, 4276 (1932).

²⁸ N. C. Beese, *J. Optical Soc. Am.*, **36**, 555 (1946).

troscopic calibration and for purposes requiring high intensity of near-infrared radiation, for instance in the quenching and infrared excitation of phosphorescence.

Neon and argon glow lamps have some uses in the laboratory. The cold-cathode type is available in a number of forms consuming from about 1/25 to 3 watts with an efficiency of about 0.3 lumen per watt and will light from the 110-volt a. c. or d. c. mains without auxiliary equipment. Greater intensities can be realized from hot-cathode lamps, which are commercially available, consuming 500 watts. A special transformer is required for this lamp. The light output of the small 3-watt lamps is adequate for the calibration of medium-size spectrographs at moderate exposures. The lines of neon are mostly in the orange and red. The 3-watt argon glow lamp emits most of its radiation in the blue, violet, and near ultraviolet, and is sometimes used for the excitation of phosphorescence.

Besides their limited use as spectral sources, neon lamps are useful laboratory adjuncts as indicators of live circuits, for testing polarity of circuits (the glow appears at the negative electrode), and for distinguishing between a.-c. and d.-c. circuits (on a. c. both electrodes appear bright). Neon lamps must be used in conjunction with a ballast resistance. In the screw-base lamps, this is provided in the base by the manufacturer but for certain types the resistance must be placed in the circuit by the user.

The *iron arc* is used as a wave-length standard in the visible and longer ultraviolet, but is feeble in lines shorter than about 2600 Å. Direct-current (110 or 220 v.) is required. In the Pfund²⁹ arc (Fig. 13), whose spectrum furnishes the international secondary and tertiary standard wave lengths, the lower electrode (+) is an iron rod about 12 mm. in diameter, carrying a bead of iron oxide in a small depression. The upper electrode (−) is an iron rod, about 6 mm. in diameter, to which is attached a brass bushing held in position by a set screw so that the rod projects about 3 mm. below. A current of 5 amp. or less is used, and a central zone 1–1.5 mm. wide from the 12–15 mm. long arc is used as the source. The bead of oxide can be formed by placing a small nail or brad in the cup before starting the arc. The electrodes are conveniently mounted in a holder permitting adjustment of the gap length during operation, and the arc is started by bringing them into contact, or by touching them simultaneously with an insulated carbon rod. A 110-v. circuit requires a resistance of 20 to 30 ohms.

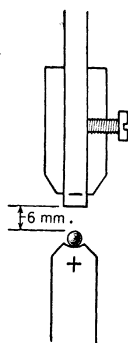


Fig. 13.—The Pfund arc.²⁹

²⁹ A. H. Pfund, *Astrophys. J.*, 27, 298 (1908).

B. ABSORPTION CELLS

Very convenient for preliminary work, for example in ascertaining best concentrations and thicknesses of solutions to be used for absorption spectra, is the Baly tube. This consists of two tubes, each closed at one end by a plane window, one of which slides within the other. The liquid under investigation is contained between the tubes, and a continuously adjustable thickness of the absorbing layer can be secured by sliding one tube with respect to the other. The liquid is admitted through a side tubule and reservoir fused to the outer tube, the volume of the reservoir being sufficiently great to contain the liquid when the windows are in contact. All-quartz Baly cells,³⁰ in which the tubes slide on the principle of the syringe, are available and are much more convenient than those with cemented windows. The manufacturers of spectroscopic equipment supply sets of cells adapted to their various spectrophotometers.

In some studies, for example in investigating the relation between absorption and concentration for strongly absorbing substances, very thin layers are required. A cell, continuously variable from 0 to 5 mm. and adjustable to 0.01 mm. by direct reading of a micrometer screw, is made by *Adam Hilger, Ltd.*, for this purpose. Other devices for use with thin layers are described in the section on infrared spectroscopy (page 1359).

C. SOLVENTS

Two obvious points must be considered in choosing a solvent: the solvent should not absorb in the same region as the solute; and the influence of the solvent on the absorption must be borne in mind.

Aromatic solvents are not suitable in the ultraviolet below 3000 Å.; aliphatic aldehydes and ketones have an absorption maximum at about 2800 Å. Carbon tetrachloride absorbs light shorter than about 2600 Å. The most transparent solvents for the ultraviolet are water, saturated hydrocarbons, ethyl and methyl alcohol, and ethyl ether.

Both the position and the structural character of absorption bands depend on the solvent. In general, polar solvents tend to shift bands to longer wave lengths, and to obliterate vibrational structure. The intensity of absorption may depend on the solvent. An inert solvent, like hexane, will produce least change in the spectral characteristics from those in the gaseous state, while the alcoholic solution will be representative of the behavior in polar solvents (Fig. 1b, page 1246). Synthetic hexane may be used as a nonpolar solvent of high ultraviolet transparency, but is expensive. Cyclohexane is useful, but may require purification from benzene.

³⁰ Obtainable from *Adam Hilger, Ltd.*, London, and *Eck and Krebs*, 131 W. 24 St., New York, N. Y.

The best commercial grade of isooctane, distilled over solid sodium hydroxide, has been recommended as being a more satisfactory hydrocarbon solvent than others available on the market.³¹ For many purposes a satisfactory solvent can be made from commercial ligroin by the process of Castille and Henri.³²

Two liters of the fraction of petroleum ether boiling from about 65° to 70° C. are shaken for twelve hours with 300 ml. of fuming sulfuric acid. After decantation, the operation is repeated twice with fresh fuming acid and then with concentrated sulfuric acid. After two washings with distilled water, the product is shaken twelve hours each with: (1) 300 ml. 10% sodium hydroxide; (2) 200 ml. 5% potassium permanganate and 100 ml. 10% sodium hydroxide; and (3) with 200 ml. permanganate and 100 ml. 10% sulfuric acid. After washing with water, the material is dried with sodium and fractionated in an apparatus with ground joints. This material will transmit radiation to about 1900 Å. Although several days are consumed in the operation, no trouble is involved other than the changing of solutions if a mechanical shaker is used.

Purification of solvents can be effected by adsorption of impurities on silica gel.³³ Petroleum ether fractions, as well as synthetic *n*-heptane, cyclohexane, and isooctane, can be purified for absorption spectroscopy in this way. About 400 g. of silica gel (Davco 659528-2000, Davison Chemical Corp., Baltimore, Md.) are placed in a glass tube about 120 cm. long and 38 to 40 mm. in diameter, constricted at the lower end. A perforated porcelain disk covered with a small plug of glass wool is held by the constriction and the top of the column of silica gel is also covered with a plug of glass wool. About 2 liters of the solvent to be purified is allowed to percolate through the column from a separating funnel, care being taken not to allow the top of the column to run dry before all the solvent has been added. The percolate is collected in fractions, the first fraction being the purest. The quality of the product should be checked spectroscopically. Usually a single passage will produce a satisfactorily transparent solvent. Any adsorbent passing through can be eliminated by distillation. 400 g. of silica gel will usually purify one to several liters of synthetic hydrocarbons, and 0.2 to 2.5 liters of petroleum ether fractions, according to the amount of impurity present. The adsorbent can be regenerated by thorough washing with water and heating in air at 325° C. for 24 hours. Removal of most of the impurities by adsorption on silica gel can be followed by chemical treatment, for example, over-night treatment with aqueous permanganate, washing and distillation over potassium hydroxide.

Grain alcohol is satisfactory as a polar solvent for qualitative purposes down to about 2300 Å. Absolute alcohol prepared by the benzene azeotropic method is contaminated by benzene. A purer alcohol can be obtained from grain alcohol by a process of Castille and Henri.³² The alcohol

³¹ T. R. Hogness, E. P. Zscheile, and A. E. Sidwell, *J. Phys. Chem.*, **41**, 411 (1937).

³² A. Castille and V. Henri, *Bull. soc. chim. biol.*, **6**, 299 (1924).

³³ M. M. Graff, R. T. O'Conner, and E. L. Skau, *Ind. Eng. Chem., Anal. Ed.*, **16**, 556 (1944).

is allowed to stand one day with one gram of iodine per liter, is fractionated, and the middle portion redistilled over a good quality of zinc dust or wool. Leighton, Crary, and Schipp³⁴ find that the common practice of drying alcohol over calcium oxide in the presence of air produces absorbing impurities, which can be eliminated by drying under nitrogen. The highest degree of purity, as measured by the absorption spectrum, is obtained by drying with aluminum amalgam.

The purification of other solvents is described in *Organic Solvents*, by Weissberger and Proskauer,³⁵ in which references to the original literature are also given. In all cases, a solvent should be tested for spectroscopic purity before being used.

D. EFFECT OF TEMPERATURE

Small variations in room temperature are usually of no great significance in the absorption spectroscopy of organic compounds; and a thermostat is normally unnecessary, although a record of the temperature of the experiment should always be made. In certain applications, for instance, the determination of the equilibrium constant between an absorbing and non-absorbing component in a system from measurements of the intensity of absorption at some specified wave length, the temperature must be carefully controlled.

Large changes in temperature may effect profound changes in absorption spectra. Of particular interest is the remarkable sharpening of the bands that accompanies the lowering of the temperature of some compounds to that of liquid air or lower. For example, sharp absorption bands appear in proteins at low temperatures.³⁶

E. ILLUMINATION OF SLIT

Usually, in the measurement of the absorption spectrum of a solution, the source must be placed some distance from the slit, and it is necessary to place a condensing lens between the source and slit in order to fill the collimator aperture with light. No advantage is to be gained by using a lens which, at its position in the optical train, subtends a greater angle at the slit than the collimator lens, since part of the light then merely strikes the wall of the collimator tube. A quartz lens of about 6 inches focal length for visible light and 1.5 to 2 inches in diameter will be adequate for

³⁴ P. A. Leighton, R. W. Crary, and L. T. Schipp, *J. Am. Chem. Soc.*, **53**, 3017 (1931). L. Harris, *ibid.*, **55**, 1940 (1933).

³⁵ A. Weissberger and E. S. Proskauer, *Organic Solvents*. Oxford Univ. Press, London, 1935.

³⁶ G. I. Northrup, J. A. Lavin, and H. S. Taylor, *J. Am. Chem. Soc.*, **55**, 3497 (1933).

most purposes. The shortening of focal length for ultraviolet light should be remembered: if for any reason it should be necessary to focus the ultraviolet sharply at the slit, focusing can be performed with the help of a fluorescent screen placed in front. (The Corning Fluorescent Canary glass filter is useful for this purpose.)

F. ALIGNMENT OF APPARATUS

Much time will be saved if the spectrograph is provided with an accurately placed optical bench fitted to the instrument, on which the stands carrying the lens and absorption tube can be rigidly placed on the continuation of the collimator axis. If one is not provided with the spectrograph, it is worth while to adapt a simple optical bench to carry the accessories desired.

The centers of the source, condensing lens, and absorption tube must be colinear with the center of the slit and with the collimator axis. The slit, of course, should be vertical. After setting the source at the correct height, its placement on the axis is achieved by looking into the part of the camera where the dispersed visible light falls, and, with a wide slit and no condensing lens, by displacing the source sideways until its image is seen diffusely in the camera lens. The source is then on the axis of the collimator. The condensing lens is now placed in the beam so as to focus the source at the slit; and if the collimator lens is filled with light, the arrangement is satisfactory. The absorption tube is placed near the slit, and any light which does not pass through the absorbing medium is prevented from entering the slit by a suitable diaphragm of cardboard.

Suitable concentrations and thickness of unknown absorbing substances are found by trial and error. The Baly tube is very useful in this connection. It is filled with solution, *e. g.*, 0.1%, and photographs of the spectrum are taken with different noted thickness until the inner and outer tube faces are practically in contact. Further exposures are made in the light of the information yielded by this preliminary trial.

Focus Plates.—A newly set-up instrument should be tested for the position of the lens which yields the spectrum of best definition. In the latest design of medium quartz spectrographs, the only adjustment that can be made is that of the collimator lens, while in other models the tilt of the camera must also be varied. A preliminary adjustment of focus can be made visually by adjusting the spectrograph lens for best definition of the lines of a mercury or iron arc on a ground glass screen in the plate holder, or, for the ultraviolet, on a fluorescent screen. The Corning Fluorescent Canary glass³⁷ is excellent for the purpose, or a coating of anthracene, will-

³⁷ *Corning Glass Works, Corning, N. Y.*

mite, etc., on a glass plate can be used. Zinc sulfide screens, although intensely luminous, are not as suitable because of the relatively lasting nature of their phosphorescence. The focusing is completed photographically. With a narrow slit (page 1262), a series of photographs of a line spectrum (*e. g.*, iron or mercury arc, in the visible and near ultraviolet, the copper spark in the further ultraviolet) is made at regularly changed and noted lens settings and the position for maximum sharpness of the lines in the center of the plate is noted. In good flat-field quartz instruments, the definition will be equally sharp over the whole plate. If the camera is provided with a tilting adjustment, the operation is repeated with the lens position fixed at the setting found for best focus at the center of the plate, with different position of tilt. The final settings are arrived at as the result of successive approximations. In these photographs, the lines must not be broadened by overexposure, and a preliminary determination of exposure time is required. The time required to photograph the spectrum of an arc focused on the slit of a medium-sized quartz spectrograph will be the order of one second.

Adjustment of Visual Spectrometers.—The only adjustments that should be necessary in a constant-deviation visual spectrometer are those of the focus of telescope and collimator lenses, and of the wave-length drum, described on page 1255. The adjustment of focus is most simply made by removing the telescope from its mount and focusing it for parallel light by viewing a distant object through an open window. The cross hairs are first brought into sharp definition by adjusting the focus of the eyepiece, and the telescope is then focused until the distant object is distinct. The telescope is replaced, and the collimator-lens setting found by illuminating the slit with a line source, such as a mercury arc, using a wide slit, and by adjusting the lens until the edge of a sharp spectral line, viewed in the telescope, shows no parallax with the cross hairs (that is, the position is retained relative to the cross hairs as the eye is moved across the eyepiece).

G. DETERMINATION OF WAVE LENGTH. HARTMANN DISPERSION FORMULA

Most makers of ultraviolet spectrographs provide the instrument with a means of registering photographically a wave-length scale on the plate used to photograph the spectrum. While these scales are inadequate for precise determinations of wave length, they are always useful in orientation, and for work on organic substances at moderate dispersion are often sufficiently accurate. They should, however, be checked, as described below, against a known arc or spark spectrum, and, if necessary, a correction table should be compiled.

Accurate measurements of wave length are made against a standard spectrum. In absorption spectroscopy, if the source has a sufficient num-

ber of fine lines in its spectrum, these can be taken as standards, careful identification of the lines being made with the help of published photographs and tables. Enlarged photographs of the spectra of iron, copper, helium, neon, and other elements are listed in the catalogues of *Adam Hilger, Ltd.*

The atlas of spectra of Eder and Valenta³⁸ is still useful in aiding in the identification of emission spectra, though the wave lengths reported there should be checked against more recent measurements. By far the most complete of wave-length tables are the Massachusetts Institute of Technology *Wavelength Tables*.³⁹ The smaller *Tabelle der Hauptlinien* of Kayser⁴⁰ and *Wave-Length Tables for Spectrum Analysis* by Twyman and Smith⁴¹ are very useful, as are the spectral tables in such standard compilations as the *International Critical Tables*, Landolt-Börnstein, and *Tables Annuelles*.

When the photographic scale is lacking or inadequate, a standard spectrum must be photographed in juxtaposition with the unknown, best with slight overlapping, in such a way that the adjacent portions of the two spectra traverse identical paths within the spectrograph. The instrument should be provided with a Hartmann diaphragm. The unknown spectrum is photographed through one portion of the slit, defined by a hole in the diaphragm, after which another portion immediately adjacent, defined by another hole in the diaphragm, is illuminated by the standard source and its spectrum photographed. The plate holder must not be moved in this operation. The standard source is focused on the slit without moving the condenser lens from the position it occupied in photographing the unknown spectrum, so that the contiguous portions of the two spectra within the spectrograph pursue identical paths, and the wave length in the unknown can be estimated by comparison with those in the standard. The position of the known lines and of the features of the unknown spectrum are measured by means of a traveling microscope, with the customary precaution against back-lash errors, of always approaching the lines from one side. If high precision in wave length is not demanded, the unknown wave length can be read off from a calibration curve of distances of known lines from some fiducial line plotted against wave length. A linear interpolation of the wave length of an unknown line from two known lines can be made when the range of interpolation in prism spectrograms is not more than a few ang-

³⁸ J. M. Eder and E. Valenta, *Atlas typischer Spectren*. 2nd ed., Akadem. Wissenschaften, Vienna, 1928.

³⁹ Edited by G. R. Harrison. Wiley, New York, 1939.

⁴⁰ H. Kayser, *Tabelle der Hauptlinien*. 2nd ed., Springer, Berlin, 1939.

⁴¹ F. Twyman and D. M. Smith, *Wave-length Tables for Spectrum Analysis*. 2nd ed., Adam Hilger, Ltd., London, 1931.

stroms; but when the standards are too sparsely distributed the *Hartmann dispersion formula* can be used. This is the relation:

$$\lambda = \lambda_0 + \frac{c}{d_0 - d} \quad (7)$$

where λ_0 , c , and d_0 are constants and λ is the wave length of the line at distance d on the plate from a fiducial line, most conveniently one of the extreme lines used in the evaluation of the constants. Three known lines are chosen, well spread over the region to be measured, the first as the point from which all measurements are made; the values of λ and d are set in the formula, and the constants λ_0 , c , and d_0 are calculated. The formula is valid for interpolation over some hundreds of angstroms, but should not be used even for a small extrapolation. The following example may facilitate the setting up of the equations for determining the constants.

The three mercury lines, 4047, 4348, and 4916 Å., were taken as standards, and all lines on the plate were measured from 4047. As measured by a traveling microscope, 4348 was 18.30 mm. from 4047 and 4916 was 40.44 mm. away. The equations are:

$$4047 = \lambda_0 + \frac{c}{d_0} \quad (a)$$

$$4348 = \lambda_0 + \frac{c}{d_0 - 18.30} \quad (b)$$

$$4916 = \lambda_0 + \frac{c}{d_0 - 40.44} \quad (c)$$

Subtracting (a) from (b):

$$301 = \frac{18.30c}{d_0^2 - 18.30d_0} \quad \text{or} \quad d_0^2 - 18.30d_0 = \frac{18.30c}{301} = 0.060796c \quad (d)$$

Similarly, subtracting (a) from (c):

$$d_0^2 - 40.44d_0 = 40.44c/869 = 0.046536c \quad (e)$$

Subtracting (e) from (d):

$$\begin{aligned} 22.14d_0 &= 0.014260c \\ c &= 1552.6d_0 \\ c/d_0 &= 1552.6 \end{aligned} \quad (f)$$

Substituting in (a):

$$\begin{aligned} 4047 &= \lambda_0 + 1552.6 \\ \lambda_0 &= 2494.4 \end{aligned}$$

Substituting in (b):

$$4348 = 2494.4 + \frac{1552.6d_0}{d_0 - 18.30}$$

By rearranging:

$$\begin{aligned} 1853.6d_0 - 1853.6 \times 18.30 &= 1552.6d_0 \\ 301.0d_0 &= 1853.6 \times 18.30 \\ d_0 &= 112.69 \end{aligned}$$

Substituting in (f):

$$c = 1552.6 \times 112.69 \quad \log_{10} c = 5.24296$$

If logarithms are used in the computation, it is unnecessary to evaluate c from its logarithm.

The constants in the Hartmann equation for this spectrum are $\lambda_0 = 2494.4$, $d_0 = 112.69$, $\log c = 5.24296$, from which by test the wave lengths of a number of lines between 4047 and 4916 Å. were evaluated and found to agree with standard values to within 0.2 Å., part of which error was undoubtedly due to errors in reading, and not to the formula.

Of standard lines, the iron arc is richest in accurately known wave lengths throughout the visible and ultraviolet to about 2600 Å.; the copper spark is useful in the shorter ultraviolet.

IV. ABSORPTION SPECTROPHOTOMETRY

The kind of spectrograms obtainable by the methods described in the last section can be of great value as an aid to identification of substances, but vastly more powerful data are provided if the intensity of absorption at each wave length is known. Quantitative analysis by light absorption is then possible, with its host of applications to general analysis and purity control, study of equilibrium of tautomers and other reactants, and rates of reaction. Even in the matter of identification it is important to acquire some knowledge of the amount of absorption, for both wave length and intensity of absorption are characteristic of the compound, or of functional groups in the molecule (see page 1393). If, *e. g.*, a compound should show an absorption band at the wave length corresponding to a functional group but with a highly abnormal intensity, the characterization of the compound as containing the group would be rendered very doubtful unless adequate environmental influences for the abnormality could be adduced.

1. Laws of Absorption of Light. Definitions

A. LAMBERT'S LAW

A parallel beam of monochromatic light is weakened in intensity by absorption in passing through a homogeneous absorbing medium in such a fashion that the intensity diminishes in geometrical progression as the thickness increases in arithmetical progression; or, more succinctly, the inten-

sity diminishes exponentially with the thickness. Let I be the intensity to which the incident intensity, I_0 , of a parallel monochromatic beam is reduced after passage through a thickness, d , of absorbing medium, then:

$$I = I_0 e^{-kd} \quad (8)$$

where k , the absorption coefficient, is constant for a given wave length, but is, in general, a function of wave length and temperature, and e is the base of the natural logarithms. An equivalent statement is that any layer of given thickness of the medium absorbs the same fraction of the radiation incident on the layer.

Equation (8) can be expressed logarithmically, to the base 10:

$$\log I/I_0 = -Kd \quad (9)$$

$$\text{or:} \quad \log I_0/I = Kd \quad (10)$$

where $K = k/2.303$, is usually termed the extinction coefficient.

The ratio I/I_0 is the fraction of the incident light transmitted by thickness d of the medium; its reciprocal, I_0/I , is the opacity, and the decadic logarithm of the opacity is called the (optical) density of the medium, D :

$$D = \log I_0/I \quad (11)$$

For example a medium with a density of 1 for a given wave length transmits 10% of the incident light at the wave length in question. The term "extinction" is also sometimes used for density.

Within the range of intensities experimentally available, these laws are universally true for pure substances or solutions. Experimentally, apparent deviations from Lambert's law may be found, but they can almost always be attributed to factors like inadequate spectral resolution in regions in which the absorption coefficient changes very rapidly with wave length, or to the presence of spurious radiation of different wave length from that for which the instrument is set (p. 1327). But while the absorption and extinction coefficients of pure homogeneous liquids and, with due consideration of directional dependence, of pure solids, are characteristics of the substance (the extinction coefficient being the density of unit thickness), this is obviously not so for solutions and gases, in which the light transmitted by unit thickness depends on the concentration.

B. BEER'S LAW

In the simplest case, in which the molecules of an absorbing solute act independently of each other, and in which the influence of a colorless solvent is constant with concentration, each molecule of solute absorbs the same fraction of the light incident on it; that is, the fraction of the light trans-

mitted by a given thickness of solution decreases exponentially with increasing concentration of solute, or the density increases linearly with the concentration. Combining the effects of concentration and thickness, one has, according to Beer's law, for monochromatic light:

$$I = I_0 e^{-k_m c d} \quad (12)$$

where c is the concentration, in moles per liter, d is the thickness in centimeters, and k_m is the molar absorption coefficient, a characteristic of the solute, independent of its concentration but depending on solvent and temperature. Experimental results are usually reported in terms of the molar extinction coefficient ϵ , defined by the relation:

$$\log I_0/I = \epsilon c d \quad (13)$$

or:

$$\epsilon = D/cd \quad (14)$$

the concentration being expressed in moles per liter and d in centimeters. In the same way, the specific extinction coefficient $K_{sp.}$ is defined by the relation

$$K_{sp.} = D/c'd \quad (15)$$

where c' is the concentration in grams per cubic centimeter.

To test the applicability of Beer's law, the densities of solutions of the same thickness (corrected for loss of light at reflecting surfaces) are plotted against the concentration; a straight line passing through the origin shows Beer's law to be obeyed, the slope being equal to the product ϵd . It is very often obeyed. Apparent exceptions may arise from inadequate spectral resolution or from the presence of spurious light, but true deviations also occur. These indicate molecular interactions, such as those causing association, dissociation, complex formation, or change in solvation.

In plotting intensity of absorption as ordinates against wave length or wave number as abscissae, the molar extinction coefficient is the best function to represent the intensity, as it is independent of concentration (if Beer's law holds) and thickness, while the shape of the percentage transmission-wave-length curve depends on the concentration and thickness. If the range of molar extinction coefficients is very great, the logarithms of the quantity may be plotted against wave length. If an absorbing substance is present in unknown concentration, the extinction coefficients cannot be computed from the density; in these cases it is useful to report results as logarithms of the density against wave length, since all such curves for a given substance obeying Beer's law run parallel to each other, that is, the shape is independent of concentration and thickness.

Equation (14) is the basis of quantitative analysis by absorption spectrophotometry; a substance of known molecular extinction coefficient ϵ , obeying Beer's law, which has an optical density D at thickness d , has a concentration of $c = D/\epsilon d$.

C. ABSORPTION OF MIXTURES

In a mixture of independently absorbing substances, the extinction coefficient is additively composed of those of the components: a solution of concentrations c_1, c_2, \dots of noninteracting substances of molar extinction coefficients $\epsilon_1, \epsilon_2, \dots$ placed in a cell of thickness d absorbs light according to the equation:

$$\log I_0/I = (\epsilon_1 c_1 + \epsilon_2 c_2 + \dots)d \quad (16)$$

If the extinction coefficients of two absorbing substances in a solution are known, their concentrations can be determined by absorption measure-

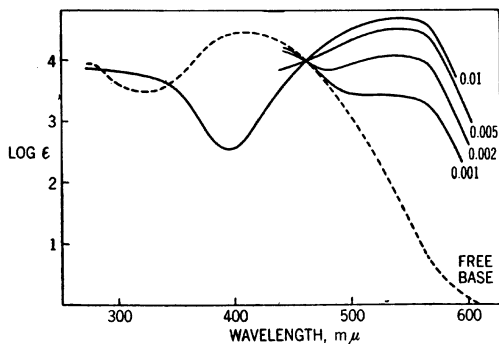


Fig. 14.—Isosbestic point for two compounds in equilibrium: 0.00025 *M* methyl yellow in the presence of trichloroacetic acid at concentrations denoted at each curve.

ments at two wave lengths, and similarly n independent components can be determined by solving the n simultaneous equations for the densities at n wave lengths (see page 1387, and Chapter XXII, page 1430).

It frequently happens that compounds existing in equilibrium have overlapping absorption bands, their curves of extinction coefficient *vs.* wave length intersecting at λ_i . If two such compounds, *e. g.*, tautomeric forms, each obeying Beer's law, are in equilibrium, then, at constant temperature and constant total and total molar concentration and constant thickness of layer, the density-wave-length curves of all equilibrium mixtures will inter-

sect at the fixed wave length λ .⁴² This fixed wave length of intersection has been called the *isosbestic point*. The experimental realization of this situation is a criterion for the existence of two compounds in equilibrium. This often happens for the different colored forms of indicators, for instance, for the yellow and red forms of methyl orange, or methyl yellow, or for some of the forms of dyes like thionine.⁴³ Figure 14 shows the absorption curves of methyl yellow in benzene for various additions of trichloroacetic acid.⁴⁴

2. General Principles of Absorption Spectrophotometry

The object of absorption spectrophotometry is to evaluate the intensity of an absorption spectrum, by expressing the molecular extinction coefficient of the absorbing substance as a function of wave length. In all precise methods, the absorbing substance is substituted for a "blank" in the same, or in an equivalent, beam. The energies in the beam containing the blank and that containing the absorbent may be measured directly, or that of the blank beam may be weakened in a measurable way until it equals that of the beam containing the sample. This measurement or comparison is performed by the use of some kind of radiometer or photometer; another part of the instrument, a monochromator or spectrograph, isolates a narrow wave-length range for photometric observation, the whole forming a spectrophotometer.

In single-beam photometry of solutions, two identical cells, one containing the solution, the other the solvent, are introduced alternately into the beam, which comes from a source of constant intensity. In double-beam photometry, a single source is accurately divided into two equivalent beams, one passing through the solution, the other through the solvent; the diminution in intensity of the beam passing through the absorbing medium is compensated for by some device which equalizes the intensity of the blank beam to that of the other, the two beams being brought to juxtaposition in a photometric field. It will be observed that single-beam photometry requires a constant light source; variations in the intensity of the source in divided-beam photometry are without effect, but as the demands on perfectly adjusted precise optics are high it is not easy to improvise a divided-beam spectrophotometer. A stigmatic spectrograph or monochromator is required in the divided-beam method.

As detectors of the radiation, the eye, the photographic plate, the thermopile, and photoelectric cells are chiefly used.

Various methods of altering the intensity of the "blank" beam are em-

⁴² F. Weigert, *Ber.*, **49**, 1525 (1916).

⁴³ L. F. Epstein, F. Karush, and E. Rabinowitch, *J. Optical Soc. Am.*, **31**, 80 (1941).

⁴⁴ A. Weissberger and K. Fasold, *Z. physik. Chem.*, **A157**, 65 (1931).

played, including rotating sectors of variable aperture, variable slits, neutral tinted wedges, wire screens of known transmission, or optical means depending on polarizing the beams.

A. VISUAL SPECTROPHOTOMETERS

A frequently used principle of photometry is to pass two similar beams through the absorbing medium and a blank, *e. g.*, the solvent, and then to secure from them two beams linearly polarized in mutually perpendicular directions. The polarized beams, of unequal amplitude, if the specimen and blank absorb differently, then pass through an analyzing nicol prism, mounted in a divided circle in such a way that its "zero" position and the 0° and 90° graduation marks coincide with the vibrational directions of the incident beams. The analyzer is turned through an angle α from its zero position until the brightness of the two beams in the photometric field is the same; the ratio of intensities in the beams issuing from the absorption cells is then,

$$I_1/I_2 = \tan^2 \alpha \quad (17)$$

As an example of the mode of action of such instruments, figure 15 gives a sketch of the Martens spectrometer as used in the Bausch and Lomb Visual

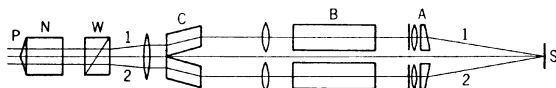


Fig. 15.—Light path in Bausch and Lomb visual spectrophotometer.

Spectrophotometer. Two equivalent beams, 1 and 2, from source *S*, a ground-glass window illuminated by a tungsten filament lamp, are selected by the wedges, *A*, collimated by the wedges and lenses, pass through cells *B* (which may be arranged vertically, with the use of the necessary reflecting prisms), and are brought together by rhombs *C* to enter the Wollaston prism, *W*. The beams from the Wollaston enter the analyzing nicol, *N*, mounted on a circle, and the biprism, *P*. Of the series of images formed by the Wollaston and biprism, two, polarized mutually perpendicularly before entering the Nicol, one from beam 1, the other from beam 2, are focused in juxtaposition at the slit of a constant deviation spectrometer, the dividing line being the edge of the biprism. The observer sees two adjacent spectra in the telescope eyepiece, one of which is from the beam which has passed through the solution, the other from the beam traversing the blank. The analyzing Nicol is turned until the spectra appear equally bright. The extent of spectrum viewed is controlled by a slit in the eyepiece—it

should not show any change in hue across its breadth, but must be broad enough to allow comparison of the upper and lower images. It will be observed that, in this arrangement of juxtaposed spectra, the second slit does not influence the spectral purity but only the extent of spectrum viewed: wave length varies over the second slit and the position of absorption bands can readily be seen. Within the limit that there be no great variation in color or intensity across the second slit, its width can be adjusted independently of that of the entrance slit to suit the rate of variation of absorption with wave length from point to point in the spectrum. The polarization spectrophotometers of Hilger and of Bausch and Lomb use this type of photometric field. Certain other photometers employ an optical system which gives a field of uniform color, of a spectral purity depending on the ocular slit as well as the entrance slit. Independent adjustment of the slits beyond narrow limits cannot be made in this case, and the two slits should be arranged to contain equal wave-length intervals. Slit-width errors are considered on page 1303.

In the Bausch and Lomb instrument, two quadrants of the circle are divided in degrees, one directly in transmission, and one directly in density. The accuracy of the transmission and density scales depends on exact orientation of the vibrational directions of the polarized beams from the Wollaston with the directions of the 0° and 90° divisions and the zero point of the Nicol. When both beams are clear, match must be secured at 45° .

The best way of taking readings is to place two similar cells in the beams, one containing solution, the other solvent, to find the position of equality, averaging several readings, and then, after interchanging the cells in the beams, to repeat the operation. The effects of accidental inequalities in the intensity of the two photometric beams and of any absorption by the solvent are thereby eliminated. Let α_1 and α_2 be the readings for equality of the beams in the two cases and T_x and T_s the transmissions of solution and solvent, respectively, then:

$$T_x/T_s = \tan \alpha_1 / \tan \alpha_2 = \tan \alpha_1 \cot \alpha_2 \quad (18)$$

Let I_1 and I_2 be the intensities of the two beams incident on the cells, T_1 and T_2 the fraction of incident light transmitted by the front and rear windows of the two absorption cells, T_s the fraction transmitted by the solvent, and T_x by the solute. Then the cell containing the solution transmits a fraction $T_x = T_1 T_s T_2$ of the incident light and that containing the solvent $T_s = T_1 T_s T_2$. When the solution is in the beam of intensity I_1 and the solvent in the other, the position of the Nicol for equality is given by:

$$I_1 T_x / I_2 T_s = \tan^2 \alpha_1 \quad (19)$$

On interchange:

$$I_1 T_s / I_2 T_x = \tan^2 \alpha_2 \quad (20)$$

whence:

$$T_x/T_s = \tan \alpha_1 / \tan \alpha_2 \quad (21)$$

Since the reading with the blank cell will change with wave length, the interchange should be carried out at every wave length setting.

If, in the interest of speed, the method of interchange of cells is not used, a tolerable correction for inequalities in the photometric beams can be made by matching the solvent and sample cells filled with pure solvent. A "zero correction" can thus be found, which will hold provided the same cells are always used for solvent and sample, respectively. This correction should be determined throughout the spectrum, although its variation with wave length is often found to be very small.

Precision.—Under favorable conditions, the eye can detect differences of brightness of about 1% in the parts of a photometric field, irrespective, within limits, of the absolute brightness. In the extreme red and violet, the precision of estimation is lower. The corresponding errors in the density and in quantities proportional to the density are the smaller the greater the density, amounting to about 0.4% at density 1 (10% transmission) to about 4% at density 0.1 (79% transmission). Normally, best results are obtained between densities of 0.3 and 1 (50 to 10% transmission). See Chapter XXII, page 1407.

Concentration and Thickness.—A fairly concentrated solution is prepared with the usual precautions of analytical chemistry, from which a series of different dilutions is made in volumetric flasks. A common cause of error in the value of the extinction coefficient is the presence of water or other volatile material which, unless removed by suitable drying, can easily amount to several per cent of the weight of some organic powders. The presence of dust, particles of filter paper, etc., introduces errors through scattering. The approximate position of the maximum is found and a concentration and thickness chosen so that the density about the maximum is about 1. The thickness should preferably be great enough for the error in thickness to be insignificant. Various concentrations should be measured, and, if there is no deviation from Beer's law, values of the molecular extinction coefficient at the different concentration should agree within about 2%.

High Densities.—Densities higher than about 2 are difficult to measure directly because of the low brightness and the rapid variation of the tangent with angle for large angles. The difficulty can be overcome by measuring the density of the sample as compared with that of a filter of the same hue or of a neutral hue; the density of the comparison filter is then measured with respect to a transparent glass plate (to compensate for loss by reflection). If d is the density of the sample with respect to the filter and d_f the density of the filter with respect to its colorless compensator, then

d_s , the density of the sample, is equal to $d + d_s$. With a sufficiently intense source, densities to about 4 can be measured with fair accuracy in this way.

Effect of Slit Width.—The limitations on spectral purity imposed by diffraction at the apertures of the optical system have already been discussed in connection with resolving power (page 1260). When a monochromator with wide slits is used to isolate spectral regions from a continuous spectrum, the radiation transmitted by the exit slit is obviously heterogeneous to an extent depending on the slit width and the dispersion of the instrument. In order to secure enough energy for the purpose in hand, monochromators in spectrophotometry and other applications are frequently used with slit widths so wide that the impurity caused by diffraction is small compared with that caused by the change in wave length across the geometric image.

We shall discuss the impurity itself and its effect on resolution, and then its effect on the values of extinction coefficients. Consider first the passage of radiation through the exit slit of a monochromator whose entrance slit is illuminated by monochromatic radiation, say the mercury green line, $546\text{ m}\mu$. For simplicity let the second slit be just as wide as the image of the first in the plane of the second; if, as is commonly true, the focal lengths of the collimator and camera lenses are the same, the geometric widths of the two slits are then the same. Let the slits be 1 mm. wide and let the dispersion of the instrument be $5\text{ m}\mu$ per mm. The slit positions are fixed and the spectrum is scanned by turning the prism. When the prism is set at $546\text{ m}\mu$ the image of the entrance slit will just fill the exit slit with green light (B, Fig. 16a). As the prism is turned from the long to short wave lengths, green light will just begin to enter the exit slit when the prism setting is $5\text{ m}\mu$ longer than the true setting for the line (C), and will just leave the other side of the exit slit at a setting $5\text{ m}\mu$ shorter than the true setting (A). If the energy transmitted by the slit as a function of prism setting is measured by a thermopile, a curve like that of figure 16b will be obtained.

In the case considered there is no spectral heterogeneity in the light

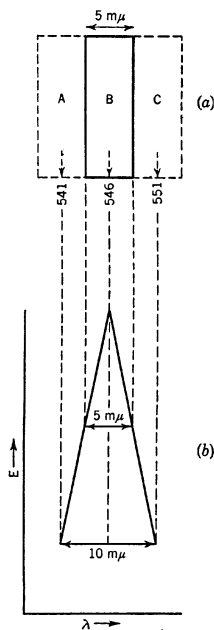


Fig. 16.—Effect of slit width on the energy-wave-length setting relation for a monochromatic line.

transmitted by the slit, since only a single wave length is supposed to illuminate the entrance slit: rather there is a wave-length impurity in the sense that some light of wave length $546\text{ m}\mu$ is transmitted at other wave-length settings. If the problem now is the isolation of regions from a continuous spectrum, we see that at the setting $546\text{ m}\mu$ the light transmitted through the second slit comprises all wave lengths between 541 and $551\text{ m}\mu$, a spectral range of $10\text{ m}\mu$, and similarly at other settings. The spectral slit width in wave-length or frequency units is defined as half the wave-length or frequency range transmitted by the instrument. Two monochromatic beams of equal intensity separated by the spectral slit width will just not be resolved by the instrument.

The possibility of inadequate resolution makes it advisable to include a statement of the spectral slit width in published reports on extinction coefficients along with the other data normally considered relevant to the estimate, such as solvent, temperature, concentration, and cell thickness. This practice has long been prevalent among infrared workers, who were early made aware of the merely domestic significance of transmission measurements on incompletely resolved bands. If this information is available, results on poorly resolved bands can be utilized in other laboratories if the same resolution is used.

The spectral impurity introduced into the measurement by the use of finite slits causes the transmission determined at any wave-length setting to be only an average for the spectral region included by the slits. Where the variation of absorption coefficient over a slit width is small, the error is small, but otherwise serious errors arise; the measured absorption coefficients are lowered at the maxima and raised at the minima from the true values. The errors are approximately proportional to the square of the sum of the entrance and exit slit widths, and to the second derivative of the portion of the intensity-wave-length curve included by the slits.⁴⁵ Corrections are laborious to make and the better procedure is to measure at various slit widths until further narrowing causes no significant changes in the value of the density. No general value can be given for slit width, but as an orienting figure 0.2 to 0.5 mm. will normally be satisfactory in visual spectrophotometry of substances with absorption bands of "half-width" characteristic of an average dye, say about $40\text{ m}\mu$.

For narrower bands, such as occur in some benzenoid hydrocarbons in nonpolar solvents, considerably narrower slit widths may be required. Table V from the work of Hogness, Zscheile, and Sidwell,⁴⁶ shows the effect of slit width on the extinction coefficient at a minimum and at a maximum, respectively, in the spectrum of benzene. The measured extinction coef-

⁴⁵ E. P. Hyde, *Astrophys. J.*, **35**, 237 (1912).

⁴⁶ T. R. Hogness, F. P. Zscheile, Jr., and A. E. Sidwell, *J. Phys. Chem.*, **41**, 413 (1937).

ficient decreases at the maximum and increases at the minimum as the slit width increases, both approaching the same value at very wide slit widths when resolution of the individual bands is no longer effected. The smaller effect of changing slit widths on the apparent extinction coefficient of the minimum than on the maximum of absorption is due to the relatively greater sharpness of the maximum than of the minimum.

TABLE V
EFFECT OF SLIT WIDTH ON APPARENT EXTINCTION COEFFICIENT^a

Slit, mm.	Wave-length range transmitted, Å.	Apparent extinction coefficient	
		2525 Å. (min.) ^a	2540 Å. (max.) ^b
0.07	5.6	45.3	212
0.09	7.2	45.1	212
0.11	8.8	45.3	204
0.13	10.4	45.2	202
0.16	12.8	45.2	199
0.20	16.0	45.5	190
0.30	24.0	47.0	170
0.40	32	50.8	154
0.50	40	52.0	136
0.70	56	57.2	118
1.00	80	62.5	104
1.50	120	69.0	89
2.00	160	72.2	77

^a 0.0064 *M* solution in iso-octane; 1-cm. cell.

^b 0.0016 *M* solution in iso-octane; 1-cm. cell.

To find the wave-length interval covered by the width of the entrance slit, the slit is illuminated by an atomic line source, *e. g.*, a mercury arc, furnishing isolated lines which are monochromatic to a small fraction of an angstrom unit. With an entrance slit of given width, the spectral image of the line is caused to traverse the eyepiece, and the scale drum positions for the edges of the line noted as they pass the cross hair or a narrow exit slit. Half the difference between the apparent wave lengths corresponding to the edges of the line is the slit width in wave-length units. With prism instruments, this should be done at various wave lengths, for which the mercury spectrum, with the addition of a red line, *e. g.*, the red hydrogen or lithium lines, is convenient. In the ultraviolet, with photoelectric spectrophotometers, the spectral slit width for various geometric slit widths can be determined from the procedure sketched in figure 16 (page 1303).

The step-by-step determination of extinction coefficients throughout a spectrum by subjective visual spectrophotometry is at best a tiring operation, and certain common-sense precautions must be taken to secure accuracy. A well-ventilated darkened room free from extraneous light should be used; manipulation of the wave length and photometric scales should

be convenient; and the scales should be adequately illuminated by shielded lights. At every wave-length setting, a number of photometric readings should be made and averaged; a number of readings made one immediately after the other lead usually to higher precision than a few made with long periods of viewing. Occasional checks of the wave-length calibration should be made, for instance by observing the maxima of absorption in "didymium" glass or other well-defined absorption bands of known wave length or, probably better, by noting the position of the lines of the mercury arc. Besides polarizing devices, other means can be used to equalize the beams in visual spectrophotometry by the two-beam method, but since most of them are described in the following section as applied to photographic spectrophotometry, further specific reference will not be made to visual applications.

B. PHOTOGRAPHIC ABSORPTION SPECTROPHOTOMETRY

Until recently, most absorption spectrophotometry in the ultraviolet was carried out photographically, although the introduction of convenient photoelectric spectrophotometers is now rapidly replacing the photographic method. In essence the following two methods may be used in photographic photometry: (a) on the same photographic plate, the density of a portion which has been exposed to a beam passing through the absorbing medium is compared with that of a portion exposed for the same time to the same or an equivalent beam passing through a blank; or (b) the intensity of a beam passing through the blank may, by appropriate means, be diminished so that the density it produces in the plate equals that yielded in the same time by an equivalent beam passing through the absorbing medium. The photometric principle in method *b* is that equality of photographic density in adjacent positions of a uniformly processed plate exposed for the same time to light of the same wave length indicates equality of intensity of the exciting radiation. No density-intensity calibration of the plate is required in *b*; and as the determination of density equality of the sample and blank beams can be, and usually is, made visually, no densitometer is required. In method *a*, the plate containing the densities to be compared requires a set of calibration marks produced by beams of known relative intensity, from which the density-intensity curve for the plate can be made for the wave lengths of interest. The actual densities must therefore be measured by means of a microdensitometer (see page 1312).

Most of the commercial photographic spectrophotometers are divided-beam instruments based on the method of equal densities, which differ from each other in the means used to change the intensity of the beam passing through the blank. They consist essentially of a photometer in series

with a quartz spectrograph arranged to produce two adjacent spectra at the photographic plate, one of the blank beam, the other of the sample.

Sector Photometers.—In these, the light in the blank beam is varied in a known way by a rapidly rotating sector of variable aperture. A rotating sector of fixed aperture may be placed in the sample beam. It will be observed that points of match in the two spectra are not produced strictly by equal times of exposure to equal intensities; while the spectrum of the sample is produced by a beam weakened by absorption at constant times of exposure (which may take the form of a series of flashes if a fixed aperture sector is used), the spectrum of the blank is produced by a beam of undiminished instantaneous intensity incident on the slit for shorter periods than the other beam. The principle of the rotating sector is unexception-

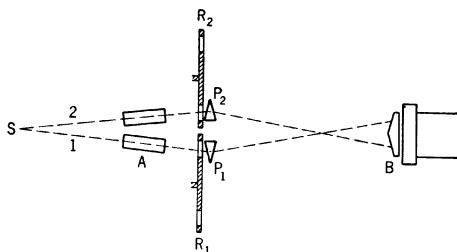


Fig. 17.—Hilger sector photometer.

able for visual observation if the period of interruption is great enough to eliminate flicker, being embodied in the well-tested Talbot's law stating that the apparent intensity of a constant source visually viewed through a rapidly rotating sector of aperture θ° is $\theta/360$ of the source viewed directly. In photography, two possible sources of error exist *a priori* in the use of the sector: (a) the reciprocity failure of the photographic plate, that is, the circumstance that, in general, the photographic effect of light is not determined merely by the total amount of luminous energy incident on the plate, measured by the product of intensity and time of exposure, but depends on the intensity level; and (b) the "intermittency" effect, the failure of the plate to integrate a set of intermittent exposures so as to yield a response equal to that produced by a single exposure of the same intensity and same total time. As a result of the fundamental studies of Webb⁴⁷ into the nature of the reciprocity and intermittency effects and of numerous specific studies of the use of sector photometers, it appears that, with sufficiently rapid interruption, the rotating-sector photo-

⁴⁷ J. H. Webb, *J. Optical Soc. Am.*, **23**, 157 (1933).

graphic method will yield accurate values of transmission. O'Brien and coworkers⁴⁸ find exact compensation between reciprocity failure and intermittency effect at the intensities normally used in spectrophotometry at frequencies of interruption above about 10 per second.

As many of the results on absorption spectrophotometry reported to the present time in the ultraviolet have been obtained with rotating-sector photometers, we shall give a short description of the sector photometer of *Adam Hilger, Ltd.* As may be seen from figure 17, light from source *S* pursues the two paths, 1 and 2, beam 1 passing through an absorption cell, *A*, containing the medium under investigation and a rotating sector, *R*₁, of fixed aperture. A wedge prism, *P*₁, directs beam 1 so that it falls on the upper half of biprism *B*, which directs the beam into the slit of the spectrograph parallel to the collimator axis. Beam 2 pursues a similar path through a cell containing pure solvent and through the variable aperture of rotating sector *R*₂, divided to read optical densities, and enters the lower part of the slit. A spectrogram of the source will contain two spectra in juxtaposition, one formed by beam 2, uniformly reduced in intensity by *R*₂, the other formed by beam 1, reduced at the wave lengths absorbed by the medium. At certain wave lengths, the photographic density of the two spectra will be equal; at those wave lengths, the absorption of the medium corresponds to the density indicated by the setting of the variable sector. By photographing in this way a number of spectra with different sector settings, the absorption spectrum of the medium can be mapped.

A tungsten-steel spark is usually employed as source. The slit width of the spectrograph should not be less than about 0.03 mm., since it is difficult to judge the match of lines that are too narrow. Fine-grain plates of high contrast and medium speed are best. An exposure time of about ten seconds with the sector set at zero density will be about correct, and as the sector is closed to give higher densities the exposure time should be increased in approximately the ratio of the transmission at zero density to that at the increased density, *i. e.*, in the ratio of antilog *D*, where *D* is the density corresponding to the sector setting.

The Baly tube is convenient for determining the thickness and concentration of solution to use in the final measurements. The sector is set for a density of 1.5, and a thickness and concentration of solution found which will make the solution spectrum at the maxima of absorption slightly more dense than that through the variable sector. The correct concentration having been found for the length of absorption tube decided on, a series of photographs is taken at density intervals of 0.1. After processing, the plate is examined on a viewing stand by means of a lens and the lines of equal

⁴⁸ B. O'Brien *et al.*, *Phys. Rev.*, **33**, 640 (1929); **37**, 471 (1931); **41**, 387 (1932).

intensity in each pair of juxtaposed spectra marked with a dot. The wave lengths of these lines are determined from the scale or by the methods described on page 1292. In any one pair of spectra, the marked lines are the wave lengths at which the density of the solution is the predetermined value given by the corresponding sector setting; division of the density by the product of the molar concentration and thickness gives the molecular extinction coefficient.

In the use of rotating sectors, stroboscopic effects arising from a synchronization of the fluctuations of intensity of a light source fed by alternating current with the frequency of rotation of the sector must be guarded against. Such effects do not occur with high-frequency condensed spark discharges, but may occur with a hydrogen lamp driven by a 60-cycle transformer. The ordinary form of high-voltage hydrogen tube is also unsuitable for photographic spectrophotometry, because of intensity variations across the capillary, without some device permitting rapid passage of the image of the source over the area of the photometric apertures. A rapidly rocking mirror causing the apertures to be effectively uniformly illuminated for exposures over twenty seconds is made by *Adam Hilger, Ltd.* for this purpose.

The low-voltage direct-current concentrated hydrogen sources of the Allen type (p. 1275) would seem to be free from these disadvantages.

Spekker Spectrophotometer.—The Hilger Spekker Spectrophotometer avoids the rapidly moving parts of the rotating-sector instrument by controlling the intensity of the photometric beams in a divided-beam arrangement by means of a variable diaphragm. This instrument is superior to the sector photometer in the range of optical densities measurable (up to 2) and in the accuracy of the extinction settings, especially at high values of extinction.

Notched Echelon Cell Photometer.—The type of instrument just described is not well adapted for the examination of very unstable or photosensitive substances because of the time required to obtain the exposures necessary for an evaluation of the absorption curve. The automatic recording photoelectric instruments of Hardy and of Harrison and Bentley to be mentioned below require quite short times for the complete mapping of an absorption spectrum; and there is also available in the notched echelon cell of Hilger a photographic means of rapid spectrophotometry yielding the necessary data with a single short exposure with no more than about 0.4 ml. of solution. Figure 18 illustrates the light path from source *X* through the instrument to the spectrograph slit, *S*. The quartz cells, *N* and *P*, are rhomboid to permit the necessary reflections, and the troughs containing the liquids are cut in steps so as to interpose different thicknesses of absorbing medium in the beam, parallel to the

long axes of the cells. The absorbing substance is placed in *N* and the solvent in *P*. The edge of *N* nearer the slit is provided with a series of notches, and the two cells are so disposed that the slit is illuminated with light in alternate strips that has traversed equivalent paths through solvent and solution, the thickness corresponding to each pair increasing from the bottom of the slit upwards. In the path of the solvent beam is placed rotating sector *R*, set to some definite value of the density. There are thus photographed at a single exposure ten pair of spectra, one of each pair having passed through the absorbing solution, the other through the solvent

and reduced in intensity by a known amount by the sector. Points of match are found in the usual way.

Wire Screens.—The experimenter who has only an occasional sample for spectrophotometry may be interested in methods which, while not comparing in convenience and precision with those using elaborate equipment, are easily improvised and capable of yielding useful results. It is assumed that a spectrograph is available. As the divided-beam principle requires optical work and adjustment of the highest precision, it is probably best in the assembling of apparatus in the laboratory to sacrifice the advantages of the divided beam, and, with a source of adequate constancy, use a substitution method in a single beam. Similar cells, containing solvent and solution, respectively, are placed alternately in

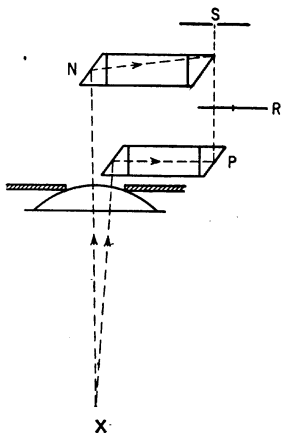


Fig. 18.—Optical path of Hilger notched echelon cell photometer (plan).

equivalent positions in the beam, and some means of changing the intensity of the beam passing through the solvent is introduced. The wave lengths of equal photographic density of adjacent spectra of solvent and solution, as formed by a Hartmann diaphragm, are found as described above (p. 1292). One of the simplest means of changing intensity is by the use of calibrated neutral wire screens, for instance, of oxidized or platinized copper gauze. A set of screens of different meshes mounted in metal rings three inches or so in diameter used in various combinations will cover a considerable range of densities. The screens should be placed near the condensing lens focusing the source on the slit and preferably should be moved irregularly by mechanical means back and forth across the beam. Calibration should be performed, for example, by a photoelectric receiver of

linear intensity response (see p. 1314) in as nearly as possible the positions occupied by the screen and photographic plate in the photometric experiments. The subject of wire-screen photometry has been discussed in detail by Harrison,⁴⁹ to whose article further reference is made.

Stücklen's Method⁵⁰.—Stücklen has described an extremely simple method of absorption photometry which, while lacking foundation on any sound optical principle, and depending rather on a fortunate accident with respect to the distribution of intensity with wave length of the hydrogen lamp and the wave-length sensitivity of photographic plates, is in fact capable of yielding values of absorption coefficients of accuracy comparable to that obtained by calibrated screens. Stücklen's observation is that, over a considerable wave-length region in the ultraviolet, the cutoff of the hydrogen continuum produced by different absorbing media occurs at the same value of the product ϵcd , the symbols representing, respectively, molar extinction coefficient, molar concentration, and thickness. An absorption spectrum is therefore made in the ordinary way by means of a high-voltage hydrogen lamp and quartz spectrograph. On the same plate as the unknown is photographed the absorption spectrum of a standard substance, for instance, potassium nitrate solution, of known concentration, thickness, and extinction coefficients. The wave lengths of cutoff of the standard spectrum are determined, and the value of ϵ at these wave lengths being known, the value of the product ϵcd for the plate can be evaluated. For the wave lengths of cutoff in the unknown, with the same length of exposure, c and d being known, ϵ can now be computed.

The method is best for absorption spectra without sudden changes in intensity, and demands a constant source, but in many trials the author has found it, using Eastman 33 plates, to give extinction coefficients within 5 to 10% of standard values.

C. CALIBRATION SOLUTIONS

In all spectrophotometric work, frequent checks of the adjustment of the photometer should be made against solutions of accurately known extinction coefficients. Aqueous potassium nitrate and potassium chromate in 0.05 *M* potassium hydroxide are suitable stable standards which obey Beer's law. The molar extinction coefficients are reproduced in table VI.^{51, 52}

⁴⁹ G. R. Harrison, *J. Optical Soc. Am.*, **18**, 492 (1929).

⁵⁰ H. Stücklen, *J. Optical Soc. Am.*, **29**, 38 (1939).

⁵¹ H. von Halban and K. Siedentopf, *Z. physik. Chem.*, **100**, 208 (1922).

⁵² G. Scheibe, *Ber.*, **59**, 2616 (1926).

TABLE VI
MOLAR EXTINCTION COEFFICIENTS OF POTASSIUM CHROMATE AND NITRATE

Wave length, $m\mu$	K_2CrO_4	KNO_3
	$\epsilon \times 10^{-3}$	ϵ
254	2.57	3.59
265	3.16	1.56
280	3.29	3.68
289	2.086	5.61
297	0.927	6.84
303	0.498	6.93
313	0.1935	5.26
334	0.985	0.45
366	4.416	..
405	1.328	..
436	0.3138	..

3. Objective Spectrophotometry

A. PHOTOGRAPHIC METHODS

Purely objective methods of photographic photometry are available if a microdensitometer is at hand for carrying out the calibration of the plate for photographic density as a function of light intensity at all wave lengths of interest. Calibration marks of the photographic density produced by known relative intensities are placed on the plate, for instance, by the use of a rotating sector, screens of known transmission, adjustable slits, or of a step tablet or a neutral wedge placed in front of the slit or plate. The wave lengths of the calibrating light must be the same as those being measured. The densities of the calibration marks are measured by means of a microdensitometer, an instrument which measures the transmission of very small areas by projecting a narrow beam through the plate on a photoelectric cell or thermopile. In figure 19, the source, *S*, a straight filament

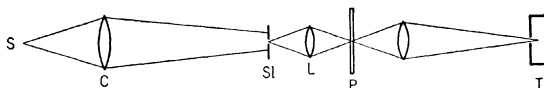


Fig. 19.—Principle of the microdensitometer.

lamp, is projected on slit *Sl* by condenser *C*. The illuminated slit is focused on plate *P* by microscope objective *L*, and the light transmitted by the plate is projected on the thermopile, or photocell *T*. As the plate is moved across the beam more or less light reaches the radiometer as the density is less or greater. The deflections of the galvanometer connected to the radiometer are therefore measures of the photographic density.

If the radiometer yields currents proportional to the intensity of the light received by it, and if the galvanometer responds linearly to the current, the density is the logarithm of the ratio of the deflections for an unexposed part of the plate to that for the exposed part. In the automatic instruments of Koch, Moll, and their followers, the drive moving the plate is synchronized with the motion of the drum on which is traced the record of the galvanometer deflections, so that there is a one-to-one correspondence between the positions on the plate and those on the galvanometer record. For many purposes, when it is not desired to measure wave lengths from the density trace, the simpler manual instruments provided by several makers of spectroscopic equipment are adequate.

Detailed discussions of the principles and methods of objective photographic photometry are in the books listed on page 1396. Specific application to absorption spectra is discussed by Moll, Burger, and Reichert.⁵³

B. PHOTOELECTRIC METHODS⁵⁴

1. **General.**—Thermopiles and photoelectric cells, of the emissive and barrier layer type, are well adapted to the measurement of radiation; spectrophotometers for the visible and ultraviolet using photocells in much the manner that infrared spectrometers have long used the thermopile are becoming increasingly important in modern practice. Although the thermopile has the great advantage of nonselective wave-length response, its relatively low sensitivity and the difficulty of amplifying its response make it less suitable than the photoelectric cell for most spectrophotometric measurements in the visible and ultraviolet.

Three different effects have been recognized, more or less suitable for the measurement of light intensity by taking advantage of an easily measurable electrical change produced by the light:

(1) The emission of electrons by metallic surfaces exposed to light *in vacuo* or in gas at low pressure under an applied electric field.

(2) The generation of current, without applied voltage, on illumination of certain systems containing a semiconductor in contact with metallic layers.

(3) A decrease in resistance of certain semiconductors on exposure to light.

The electron emission phenomenon is the basis of the phototube; the self-generative cell is illustrated by the well-known Photronic Cell and similar devices, while the photoresistive effect is made use of in selenium cells and certain sulfide cells, the best known of which is probably the Thalofide

⁵³ W. J. H. Moll, H. C. Burger, and W. J. Reichert, *J. Sci. Instruments*, 12, 148 (1935).

⁵⁴ See also Chapter XXII, pages 1411 *et seq.*

Cell. The latter effect is attended to a high degree with fatigue phenomena and lack of proportionality of the electrical effect to the intensity of the exciting light, and is rarely used in photometers for chemical analysis. Recently, improved photoconductive cells using lead sulfide and lead selenide have been developed which appear to be quite useful for photometric purposes (see page 1356).

Self-generative cells are known under a multiplicity of names: dry-disk, barrier layer, blocking layer, photovoltaic cells, photoelements. The generation of an electromotive force on the illumination of one of a pair of electrodes in an electrolyte, the long-known Becquerel or photovoltaic effect, appears to be a particular case of the phenomenon occurring in the self-generative photocell, and the name photovoltaic cell seems a suitable name for the type. Used without qualification, it will always, in this chapter, apply to the dry cell.

The currents generated by photovoltaic cells at moderate intensities of illumination are sufficiently large to actuate relatively rugged pointer microammeters in simple circuits, whereas sensitive galvanometers are required to indicate the current produced directly by the same intensity in phototubes. On the other hand, amplification by means of vacuum tubes is easy with the phototube, while the low voltage available in photovoltaic cells renders them unsuited to amplification.⁵⁵ At low intensities, therefore, the amplified phototube is much superior to the other type.

Barrier layer cells are used in some spectrophotometers, but as their main photometric use for chemists is in the measurement of the relatively high intensities occurring in colorimetry, their description is given in Chapter XXII devoted to that subject (page 1411).

2. Characteristics of Phototubes.—In the emissive type of phototube, light impinging on a metallic surface, or on a surface of metal adsorbed on its oxide, etc., causes the emission of electrons, which, after traversing a highly evacuated space, or one containing inert gas at a pressure of a few tenths of a millimeter, are collected by an electrode maintained by an external battery at a positive potential with respect to the sensitive surface.

In *vacuum cells*, in which the pressure is so low that the current from surface to collecting electrode is almost entirely electronic, the current produced by a given amount of incident light increases rapidly at first with the applied voltage, and then saturation occurs (Fig. 20, curves A). In the ideal case, the saturation currents produced by light of given wave length are proportional to the intensity, and in the more efficient commercial cells amount to 20 to 25 μ a. per lumen (p. 1267). In using the tube, it is neces-

⁵⁵ A recently described useful method of amplifying the currents from photovoltaic cells is described on page 1420, Chapter XXII.

sary to apply a voltage adequate to produce saturation at the highest intensity to be measured; 90 v. will be sufficient for most applications likely to arise. In practice, even with vacuum cells, there may be deviations from linearity of photoresponse with intensity, particularly at high voltages; and, if a method of photometry is used which depends on linearity, the intensity-current curve should be checked for the cell used. In applications demanding linearity to no more than 2%, the vacuum phototube can usually be assumed satisfactory.

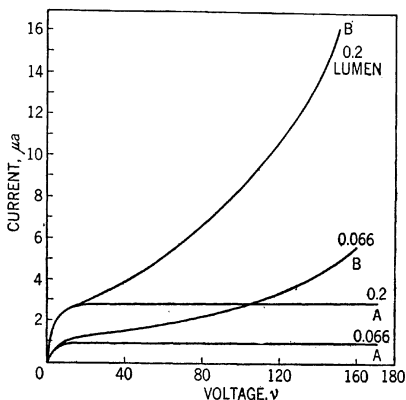


Fig. 20.—Current-voltage relations for phototubes.
A, vacuum cell; B, gas cell.

Gas cells contain usually about 0.2 mm. of argon for the purpose of increasing the primary electronic current by ionization of the gas molecules. Typical current-voltage curves for a gas cell are given in figure 20, curves *B*. At the higher voltages, the curve is concave toward the current axis, and, ultimately, with increasing voltage, a "glow discharge" is set up, the phototube acting as a gas discharge tube. This condition is to be avoided because, if prolonged, it will damage the tube, and is always accompanied by subsequent changes in sensitivity. The voltage for glow discharge is the lower the higher the intensity of the incident light. To guard against damage to a meter by the relatively high glow current should one be accidentally produced, a high resistance, of the order of a megohm, should be introduced in series with the cell and galvanometer. In amplification by means of vacuum tubes, this requirement is automatically fulfilled. For incident light flux of the order of magnitude of 0.1 lumen, 90 v. is about the maximum safe to apply to most gas cells.

Gas cells are likely to show deviations from linearity in their response intensity over large ranges of intensity. Moreover, while there is no appreciable lag in the response of a vacuum cell to the exciting radiation, gas cells have a time lag and decrease in sensitivity with increasing frequency of interruption of modulated light.

The normal increase in sensitivity of a gas cell over a vacuum cell of similar construction is about five- or sixfold at voltages safely below the glow value. Since this gain is trivial compared with that obtainable by amplification, it would seem, in general, best photometric practice to use

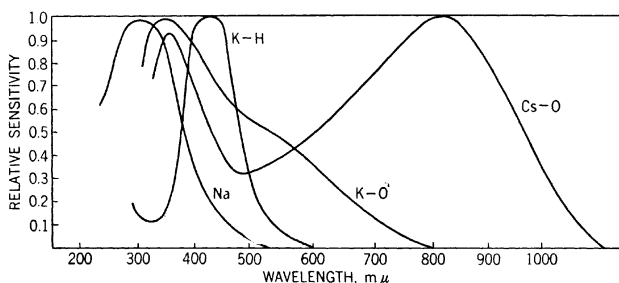


Fig. 21.—Relative spectral sensitivities of photoelectric surfaces.

vacuum cells and amplify if necessary. Borderline cases in which, for instance, the use of a gas cell might allow a simple unamplified circuit, must be considered on their merits.

The various photoemissive surfaces are selective in their spectral response. (See figure 21 in which the relative spectral sensitivity for equal incident energies of a number of surfaces is shown, but in which the different curves are not to be compared among themselves.) Cesium oxide cells have high sensitivity in the near infrared and red, a minimum at about 5000 Å., and increase again in the blue and ultraviolet. Potassium oxide cells may be used throughout the visible and lack the high infrared sensitivity of cesium cells. Potassium hydride cells are sensitive in the blue, and have very small dark currents. Sodium cells with quartz bulbs show high ultraviolet and low visible sensitivity. Cesium oxide cells in quartz envelopes can also be obtained. Thorium, titanium, and tungsten surface cells are also commercially available, with thresholds at various wave lengths in the ultraviolet. The best selection for any given purpose will have to be made from a manufacturer's catalogue, for instance, of the *RCA Manufacturing Co.*, Camden, N. J., *General Electric Co.*, Schenectady, N. Y., *The Rauland Corporation*, Chicago, Ill., and *Continental Electric Co.*,

Inc., Geneva, Ill. For direct measurement of radiation in the ultraviolet below about 3300 Å., quartz or other adequately transparent envelopes are required, but it is also possible to use a glass cell in conjunction with a fluorescent material placed in front of its window, which absorbs ultraviolet radiation and re-emits visible fluorescence. A solution of esculin in a quartz vessel, or crystals of uranium ammonium nitrate are stated by Bowen^{55a} to show a quantum yield of fluorescence independent of the exciting wave length, and could therefore be used with a photocell sensitive to the fluorescent light to form a receiver spectrally nonselective to the number of incident photons. For comparative measurements at a given wave length of ultraviolet light by means of glass photocells, the writer has found the Corning uranium glass filter "Canary Yellow" to be satisfactory for the conversion of ultraviolet to visible light.

For the measurement of absorption spectra, in which the ratio at every wave length of the light transmitted by the solution to that by a standard is the important factor, the varying spectral sensitivity of the photocell is of no importance, except insofar as it may influence the minimum usable slit width. Given adequate sensitivity and stability, linearity of response is the quality to be sought for in photocells for spectrophotometric purposes. Of the emissive types, vacuum cells are preferable in these respects, but the more sensitive gas-filled cells are usually linear at voltages not too near the glow-discharge values (that is, at voltages not greater than about 90 in most commercial cells), at the intensity levels normally encountered in spectrophotometry.

3. Amplification of Photoelectric Currents.—In the simplest circuit, comprising photocell, external battery, protective resistance, and meter in series, a fairly sensitive high-resistance galvanometer (10^{-9} or 10^{-10} amp. per mm.) is required for the accurate measurement of the currents met in colorimetry. Amplification, however, permits the use of relatively rugged instruments, as microammeters or milliammeters; and, in the measurement of small intensities such as are common in spectrophotometry or in the measurement of fluorescence, amplification is essential.

The amplification of photoelectric currents produced by phototubes depends on the manner in which they influence the potential of the grid controlling the electronic current from filament to anode in the vacuum tube. In figure 22, the more positive the potential of the grid, G , with respect to filament F , the greater is the facility with which electrons from the filament reach plate P and the greater the plate current registered by meter M . The rate of change of plate current with grid voltage, other factors remaining constant, is termed the mutual conductance of the tube. A representative

^{55a} E. J. Bowen, *Proc. Roy. Soc. London*, A154, 349 (1936).

value in these applications is 1.5 milliamperes per volt or 1.5 millimhos ($= 1500$ micromhos). If a photoelectric tube, C , is connected across the resistance, R , of, for example, 10 megohms and a photocurrent, i , is produced as the result of illumination, practically all of the photocurrent flows through R , producing a potential drop of iR across it, since the grid-filament resistance is very large compared with R . If i is 10^{-9} amp., then this potential drop is $10^{-9} \times 10^7 = 10^{-2}$ v. The grid is thus rendered 10^{-2} v. more negative than when the cell was in the dark, and the plate current decreases by $15 \mu\text{a}$. If the initial plate current passing through meter M , of the order of some milliamperes, is balanced out by a suitable circuit, B , so that the meter reads zero, then, on illumination, the photocurrent will register directly on the instrument. The resistance in the compensating circuit, B , is chosen large compared with that of the meter so as not to reduce the sensitivity by excessive shunting. The original photocurrent of

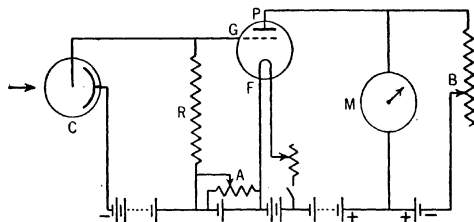


Fig. 22.—Fundamental circuit for amplification of photoelectric currents.

10^{-9} amp. has in this way produced a current of 1.5×10^{-5} amp., a gain of 1.5×10^4 , a factor which can be very considerably enlarged by suitable elaboration of the circuit.

Such a circuit has a constant amplification factor only over a restricted range of input current, since the curve of plate current *vs.* grid voltage is linear only over a small region. The circuit can be used so as to be independent of the linearity of meter readings with light intensity by compensating for the change in grid potential produced by the photocurrent. With a suitable grid bias, the plate current is balanced by the auxiliary circuit with the cell dark, as before. On illumination the meter deflects; the grid potential is then changed by the potential divider, A , until the meter again reads zero. The change of grid potential required in this operation, measured by a potentiometer or high-resistance voltmeter, is equal to the drop iR produced by the photocurrent, and furnishes a measure of this quantity which does not depend on the linearity of the amplification.

This potential change can therefore be taken as a direct measure of the light intensity, provided it is known that the photocurrents are directly proportional to the intensity and that Ohm's law holds for the resistance R .

Single-stage amplification of small photocurrents can be conveniently carried out with the so-called electrometer tubes, *e. g.*, the General Electric Company FP54, or the Western Electric Company D-96475. The balanced circuit of Du Bridge and Brown⁵⁶ is excellent for amplification of small photocurrents. A sketch of the circuit is given in figure 23.

R_0 is a bank of resistances, 10^9 , 10^{10} , and 10^{11} ohms, connected at low end to a high-insulation switch. R_1 is a 10,000-ohm rheostat, *e. g.*, RCA type 471A. R'_1 is a 50-ohm rheostat, R_2 a 2000-ohm fixed resistance, R_3 a 4000-ohm fixed resistance, R_4 a 50-ohm potentiometer, *e. g.*, RCA type 214A, R_5 a 50-ohm potentiometer, and G a galvanometer of current sensitivity of 10^{-8} amp. or more per mm.

The most critical parts of this and similar circuits are the photocell input resistance, R_0 , and the amplifying tube (or first stage in a multitube circuit), which are conveniently placed together in a well-shielded box, and attached by a multiwire cable to the remainder of the circuit, in a separate box.

Recently, circuits have been described for the amplification of small photocurrents by negative feedback amplifiers allowing the use of relatively rugged pointer instruments to measure the current.⁵⁷

The choice of amplifying circuit will depend much on the electronic virtuosity of the investigator, if he intends building his own. The circuit of Du Bridge and Brown, if constructed from good parts, will function without difficulty at first trial, without demanding very detailed experience in modern electronics. The conveniences of utilizing relatively rugged pointer instruments and perhaps of avoiding the use of batteries which accrue from more recent multitube circuits is obtained at the cost of some complexity, to cope with which a certain degree of experience in circuit analysis is an asset. This is so for the building, but not necessarily for the use and maintenance of sensitive amplifiers, and the very sensitive amplifiers provided by the manufacturers of some spectrophotometers, photometers, and fluorimeters can be operated by careful but electronically untrained personnel without difficulty.

Probably the most common source of disturbance in the use of sensitive amplifiers is electrical leakage at high-resistance places caused by the appearance of conducting films of moisture. The compartment containing the phototube input resistances and the first amplifier tube should be provided with a reservoir filled with a non-deliquescent desiccant. The "indicating" silica gel or Drierite desiccants are most

⁵⁶ L. A. Du Bridge and H. Brown, *Rev. Sci. Instruments*, **4**, 532 (1933). J. Strong *et al.*, *Procedures in Experimental Physics*, Prentice-Hall, New York, 1939, p. 418.

⁵⁷ Q. S. Heidelberg and W. A. Rense, *Rev. Sci. Instruments*, **11**, 386 (1940). R. G. Wilson, *ibid.*, **13**, 300 (1942). G. P. Harnwell and L. N. Ridenour, *ibid.*, **11**, 346 (1940).

convenient—calcium chloride or phosphorus pentoxide should not be used. The compartment containing the remainder of the circuit should also be desiccated. The condition of the desiccant should be checked first upon the appearance of unsteadiness in the meter readings. Should the desiccant condition prove to be satisfactory, a likely source of disturbance in circuits containing dry cells as sources of potential is a run-down condition of these cells.

Electrometers, as the Compton and the Lindeman types (*Cambridge Instrument Co., Inc.*, New York) can also be used for the measurement of small photocurrents.

The ease of amplification of the currents of emissive photocells recommends their use over that of photovoltaic cells for measurements of the low intensities occurring in spectrophotometry, fluorescence, phosphorescence, and the like.

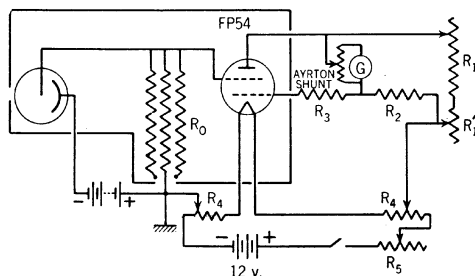


Fig. 23.—Sketch of FP54 photocell circuit.

For very low intensities, such as those conditioned by the use of monochromators with slit widths at which a close approximation to the theoretical resolving power of the prism or grating is realized, or in the study of feeble emission spectra such as Raman or fluorescence and phosphorescence spectra, the *electron multiplier tube* is preferable to the phototube and high-gain amplifier, in that the ratio of signal to background fluctuation can be made considerably smaller.⁵⁸⁻⁶⁰ The electron multiplier tube is essentially a photosensitive surface, similar to that in a phototube, and an arrangement of positively charged electrodes or “dynodes” in series (Fig. 24), the whole sealed in an evacuated tube. Photoelectrons liberated from the cathode, *C*, are directed to the first dynode, *D*₁, maintained at a positive potential, normally about 100 volts, with respect to *C*. If the electrons striking *D*₁ have acquired sufficient energy from the accelerating field, several second-

⁵⁸ C. C. Larson and H. Salinger, *Rev. Sci. Instruments*, **11**, 226 (1940).

⁵⁹ R. C. Winans and J. R. Pierce, *ibid.*, **12**, 269 (1941).

⁶⁰ R. W. Engstrom, *J. Optical Soc. Am.*, **37**, 420 (1947).

any electrons are emitted at the surface of the dynode for every primary electron striking, and these are directed toward dynode D_2 , similarly positive with respect to D_1 , where the process is repeated. In this way an increasing electron stream cascades through the tube, and is finally collected at the collector E with approximately a million-fold increase over the original photocurrent in a nine-stage multiplier, such as the RCA 931A tube, operating at 100 volts per dynode stage.⁶⁰ This current is normally applied to the input of an amplifier of moderate gain.

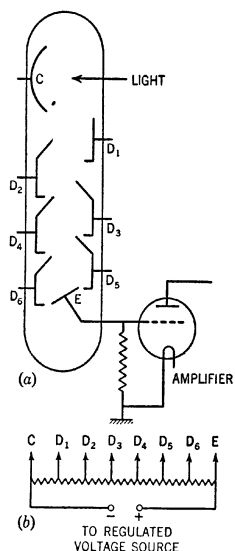


Fig. 24.—An electron multiplier tube:
(a) multiplier tube and output amplifier;
(b) means of applying voltage.

Guiding fields must be present to insure that the electrons take the proper path. In electrostatic multipliers, the accelerating fields also accomplish the focusing; in magnetic multipliers, a magnetic field must be applied for focusing purposes, in addition to the electrostatic field.

Since the emission of secondary electrons increases rapidly with the accelerating potential between the dynodes, the multiplication factor may be varied by changing the interstage voltage (normally the same for all stages), or by changing the voltage in one of the stages, with consequent defocusing of the beam. It is also evident that a well-regulated voltage supply is necessary for stable operation. The means of applying the voltages to the

various electrodes is indicated in figure 24*b*. Radio batteries could be used as the source but it is more convenient to use a stabilized supply fed from the mains (see, for example, footnotes 150 and 153).

The spectral characteristics of electron multiplier tubes are similar to those of phototubes. Tubes with a Cs—O—Ag surface have high sensitivity in the near infrared and red; the RCA Type 1P22 tube has a Cs—Bi surface with a threshold at about 7500 Å. and a maximum sensitivity at about 3600 Å.; the RCA 931 A tube has a Cs—Sb surface on a Ni base, with a threshold at about 6500 Å. and a maximum at 3600 Å.; the Type 1P28 contains the same surface in an ultraviolet-transmitting glass envelope and has high sensitivity to about 2400 Å. and a rapid fall toward shorter wave lengths caused by the absorption of the envelope.

Multiplier tubes tend to change in sensitivity during exposure, the fatigue effects increasing with the output current of the multiplier. Fatigue becomes excessive and may not be completely reversible for currents above 1 ma., and care should be taken to guard against even momentary exposure of the tube to strong light when the potentials are applied. The change in sensitivity is most rapid at the start of the exposure, and it may therefore be advisable to operate the tube for about half an hour before starting measurements. Various tests have demonstrated linearity between the output current and the light intensity, within an experimental error of a few per cent.

The lowest light intensities measurable depend on the inevitable fluctuations of dark current present in the multiplier. At dynode voltages above about 50 to 60 per stage, the dark current is due principally to thermionic emission of electrons from the cathode surface⁶⁰ which are multiplied by passage through the tube in the same way as photoelectrons. The dark current can be greatly reduced by operating at liquid-air temperature. At room temperature, the limit of detection of RCA tubes with a suitable amplifier is about 5×10^{-12} lumen.⁶⁰

Electron multiplier tubes have been applied in spectroscopy in the measurement of the line-emission spectra,⁶¹ in spectrophotometry,⁶⁵ and in the measurement of Raman spectra (page 1378). A suitable stabilized voltage supply and output amplifier are described by Jen-Yuan Chien and Bender.⁶²

In order to obtain enough energy, most photoelectric spectrophotometers made up to the present have demanded slit widths much greater than corresponds to the resolving power of the monochromator. The use of electron multiplier tubes should, however, permit a close realization of the in-

⁶¹ G. H. Dieke, H. Y. Loh, and H. M. Crosswhite, *J. Optical Soc. Am.*, **36**, 185 (1946).

⁶² Jen-Yuan Chien and P. Bender, *J. Chem. Phys.*, **15**, 276 (1947).

trinsic resolving power of the spectroscope. With the use of a multiplier tube, Harrison and Bentley⁶³ have constructed a spectrophotometer of high dispersion and high resolving power covering the spectra range from 10,000 to 2000 Å. Slit widths of 0.5 to 10 Å. are usable, and the spectra are automatically recorded at high speed.

Photoelectric Spectrophotometers.—An important feature of the use of photoelectric cells in spectrophotometers is the ease of automatic recording of the absorption curve. Hardy's instrument,⁶⁴ manufactured by *General Electric Co.*, will draw a complete transmission or extinction curve for the visible region in a few minutes, and the instrument of Harrison and Bentley already referred to will present a transmission curve from 10,000 to 2000 Å. in 70 seconds, and is capable of repeating measurements at a single wave length at intervals of 0.02 second. Such instruments, although their cost precludes their installation except in laboratories undertaking intensive programs of absorption spectrophotometry, herald a vastly increased utilization of quantitative spectroscopy; already, in the few years of its availability, the Hardy instrument has been the means of obtaining hundreds of thousands of quantitative transmission and reflection curves, chiefly in industrial laboratories, which would otherwise not have been made.

The Hardy instrument, which in its commercial form is limited to the visible spectrum, is an exceedingly ingenious combination of photometric and electronic devices much too complicated to be adequately described briefly. In essence, light from a monochromator is divided by a polarization photometer into two beams of the same wave length and state of polarization, made to fall alternately on a photoelectric cell. Equality of intensity of the beams is indicated by the circumstance that flicker is at a minimum as the beams fall alternately on the cell, and so therefore is the alternating component of the photoelectric current. If an absorbing medium is placed in one of the photometric beams, an alternating current is produced by the cell, which is amplified and transmitted to an a.c. motor whose direction of motion is determined by the relative intensities of the two beams and which alters the orientation of a prism in the photometer in such a way as to preserve photometric balance, on the attainment of which the motor stops. Connected to the arrangement is a pen whose position is determined by the orientation of the prism at balance and also by the wave-length setting. As the different regions of the spectrum issue from the exit slit of the monochromator, a record is made of the transmittance of the absorbing medium, and with suitable cams, densities, or reflec-

⁶³ G. R. Harrison and E. P. Bentley, *J. Optical Soc. Am.*, **30**, 290 (1940).

⁶⁴ A. C. Hardy, *J. Optical Soc. Am.*, **18**, 99 (1929); **25**, 305 (1935).

tances, can be automatically recorded. Only a few minutes are required to traverse the whole visible spectrum. This instrument normally uses slit widths of about 100 Å.

Precise photoelectric spectrophotometry seems to have originated with von Halban and Siedentopf.⁵¹ As an example of a modern, essentially simple and straightforward but accurate form, reference can be made to the apparatus of Hogness, Zscheile, and Sidwell.⁶⁵ Identical cells containing solvents and solution are introduced alternately into the exit beam of a quartz-prism monochromator, the receiver being a photocell whose output is amplified by an electrometer tube. Direct readings of radiation intensity transmitted by absorber and a blank are made immediately after one another, the spectrum being explored point by point by adjusting the monochromator setting. A commercial instrument of this general type is manufactured by *National Technical Laboratories*, South Pasadena, Calif., as the Beckman Quartz Spectrophotometer.⁶⁶ This covers a range from 12,000 to 2000 Å. with two photoelectric cells, a cesium oxide cell from 12,000 to 6200 Å., and an RCA C7032 cell for measurements at wave lengths shorter than 6200 Å. A hot-cathode low-voltage hydrogen lamp is used for the ultraviolet and a 32-candle power headlight lamp for the visible. The monochromator is of the Littrow type (Fig. 25), and employs an aluminized⁶⁷ spherical mirror for collimation and focusing on the exit slit. The entrance and exit slits are curved to allow for the curvature of spectral lines (p. 1252) and slit widths comprising radiation contained in a band of less than 25 to 50 Å. can be used in the visible and rather wider in the ultraviolet.

The photocurrents produced in the photocell pass through a resistance, and the resulting potential drop is balanced by a counter potential from a potentiometer, somewhat as indicated on page 1318. A rugged meter in the output of the amplifier indicates the condition of balance, and the settings of the balancing potentiometer are graduated directly in transmittance and density. Provision is made in the Beckman Quartz Spectrophotometer for the introduction of a filter in order to remove scattered visible light (see page 1327) when the prism is set for ultraviolet or far-red light. Without the filter before the exit slit, the present instruments yield a measurable response for infrared settings beyond the threshold of the caesium oxide photocell which, of course, is entirely caused by spurious light. Measurements with the Beckman spectrophotometer are dis-

⁵¹ T. R. Hogness, F. P. Zscheile, and A. E. Sidwell, *J. Phys. Chem.*, **41**, 379 (1937).

⁶⁵ H. R. Cary and A. O. Beckman, *J. Optical Soc. Am.*, **31**, 682 (1941).

⁶⁷ Rhodium-coated focusing mirrors have been found to resist tarnishing better than aluminum-coated mirrors under certain conditions of high corrosion [E. B. Clairborne, *Rev. Sci. Instruments*, **18**, 368 (1947)].

cussed by Gibson and Balcom,⁶⁸ who find it, if various precautions are taken, fully as reliable in the range from 390 to 750 $m\mu$ as other standard spectrophotometers, and much faster than visual instruments. It is pointed out that certain errors may arise in the measurement of polarizing samples; these are not inherent in the general design of the instrument, but originate in the use of crystalline quartz disks or lenses cut with the optic axis parallel to the disk faces as covers over the slits. The trouble is not present in all spectrometers made by the manufacturer, and it would be well for users to find out if their instrument shows the effect by measuring the transmission of a piece of polaroid. Spurious absorption bands will be found if the effect exists. Replacement of the quartz disks by optically isotropic covers should remove the difficulty.

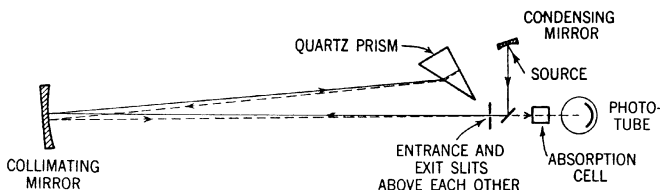


Fig. 25.—Optical path in Beckman spectrophotometer.

A photoelectric spectrophotometer using a concave replica grating, barrier layer cell, and galvanometer, usable in the range of 7500 to 3250 \AA . is made by *Central Scientific Co.*, Chicago Ill.⁶⁹ Discontinuously variable slit widths from 25 to 200 \AA . are used, and the transmittance is measured by the ratio of deflections of a box-type galvanometer with the sample and solvent, respectively, in the beam. The Coleman Photoelectric Spectrophotometer employs an amplified emissive cell, and can be used in the visible and near ultraviolet. Various models are made, one of which uses a double monochromator (page 1327) with replica transmission gratings. The photometric scale in some models is based on potentiometer readings, in a manner generally similar to that of the Beckman instrument; in others the ratio of deflections of a meter in the output of the amplifier is taken. Discontinuously adjustable slit widths from 50 to 300 \AA . are provided in some models; in others a fixed slit width of about 300 \AA . is used.

An automatic high-speed recording modification of the Beckman spectrophotometer has recently been described.^{69a} *National Technical Labora-*

⁶⁸ K. S. Gibson and M. M. Balcom, *J. Optical Soc. Am.*, **37**, 593 (1947).

⁶⁹ C. Sheard and M. N. States, *J. Optical Soc. Am.*, **31**, 64 (1941).

^{69a} T. Coor, Jr., and D. C. Smith, *Rev. Sci. Instruments*, **18**, 173 (1947).

tories, South Pasadena, Calif., have recently introduced an automatic recording spectrophotometer for visible and ultraviolet which employs electron multiplier tubes.

There is no doubt that the direct reading of transmissions and optical densities possible with photoelectric spectrophotometers permits a very considerable saving of time as compared with the quickest photographic methods, even when a step-by-step exploration of the spectrum is required. So long as absorption bands are rather broad and the change in absorption coefficient throughout a slit width is small, the accuracy of the photoelectric instruments should be no less than that of photographic, which normally is not greater than about 5% in the value of the extinction coefficient. In practice, the precision and accuracy of photoelectric estimates of transmittance are likely to exceed those of photographic estimates. With respect to the accuracy of extinction coefficients calculated from the observed transmittance, consideration must be given to slit widths and definition of the spectral image as well as to precision of the transmittance. It would seem that photographic spectrophotometers employing the best commercial quartz spectrographs could be used at narrower slit widths than most present commercial photoelectric instruments, and that, in the relatively few cases in organic chemistry where very narrow bands must be measured, greater accuracy in the absolute value of the extinction coefficient might be obtained photographically. It is true, however, that the laboratory photoelectric spectrophotometer of Hogness, Zscheile, and Sidwell,⁶⁵ employed at slit widths of 3 to 4 Å. at about 2500 Å., effected better resolution of the spectrum of benzene in solution than is shown in published data obtained with photographic instruments, as gaged by the relative magnitudes of maxima and minima in the extinction curve.

In laboratories in which numerous absorption measurements are made the greater convenience and speed of photoelectric spectrophotometers would seem to offer an almost overwhelming advantage over visual and photographic instruments for most purposes in organic chemistry. As to choice between different photoelectric instruments, much depends on the nature of the work in hand. If it is essentially spectroscopic, that is, concerned primarily with the characterization of absorption spectra as such, an instrument with great range, excellent optics, and high enough sensitivity to permit the use of narrow slits is called for. If interest is primarily analytical, a smaller variability in slit width may well suffice: reproducibility of the transmission values is the prime requisite, so that reliable empirical working curves of the density-concentration relation can be secured.

4. Spurious Radiation in Monochromators

In any monochromator, the radiation which is incident on the receiver at any setting of the prism is more or less contaminated with spurious radiation of an entirely different wave length. The causes of this contamination are various: light in the dispersed beam of other than the desired wave length may hit the sides of the housing, lens mounts, etc., and then be reflected into the receiver; lenses and prisms become coated with thin light-scattering films if exposed to laboratory air; dust particles settling on lenses, prisms and mirrors cause scattering; and the materials of prisms, lenses and gratings add their contribution of scattered light. Much may be done to minimize spurious light; housing and lens mounts may be coated with matt black paints and baffles may be introduced at appropriate places; the optical surfaces may be protected from atmospheric contamination and dust by the use of tight transparent windows over the entrance and exit slits; and optical material of the greatest freedom from flaws, bubbles, and other heterogeneities may be employed, but there still remains an irreducible minimum which it seems impossible to eliminate in a single monochromator.

The effect is particularly serious with selective receivers such as photo-cells and sources like tungsten lamps with a highly variable distribution of spectral intensity. For instance, the high sensitivity of a cesium oxide cell to red, coupled with the high intensity of red in a tungsten source, could cause measurements of transmissions in the blue to be completely fallacious if any appreciable amount of scattered or reflected red entered the cell at the wave-length setting for blue light. For the most complete avoidance of impurity in the radiation isolated by a monochromator (apart from that inherent in the use of finite slit widths), two monochromators in tandem must probably be used, as in the van Cittert double monochromator made by *Kipp & Zonen*, Delft, Holland, or the Hilger-Müller instrument made by *Adam Hilger, Ltd.*

Spurious light causes errors in measured transmissions and also causes apparent deviations from Lambert's and Beer's laws. To illustrate the effects that may arise, consider the absorption of an ultraviolet-absorbing substance as measured in an instrument 5% of whose response at the ultraviolet setting is due to unabsorbed spurious radiation of longer wave length. The radiation incident on the receiver in the absence of the absorbing medium in the beam can be put at 100 units, 95 due to ultraviolet light and 5 to spurious light. The figure of 5% is not an improbable one in practice near the ultraviolet end of the range of a receiver, where both the radiation and the sensitivity may be low. For instance, a contamination of 0.5% of longer wave-length radiation to which the receiver was 10 times

more sensitive (on an equal energy basis) than to the ultraviolet radiation would produce a response approximately 5% of which was spurious. Let the true absorption of unit thickness of the material be 50% for ultraviolet light, and let it be practically completely transparent to the spurious light. Then the true density for ultraviolet light is $\log (95/47.5) = 0.301$; the apparent density is $\log (100/52.5) = 0.280$, and the error in the density is about 7%. Let now a cell of double the original thickness be introduced in the beam. According to Lambert's law the true density is 0.602, and the true value for the transmitted ultraviolet light is obtained from the expression $\log (95/I) = 0.602$, $I = 23.75$. The apparent density is $\log (100/28.75) = 0.541$, an error of 10%. The ratio to the apparent density at unit thickness is only 1.93, and Lambert's law is apparently violated; a similar apparent violation of Beer's law occurs. Only if the spurious light is absorbed to the same extent as the true light will there be no error in the measured density; otherwise there will be positive or negative errors, respectively, as the absorption for the spurious light is greater or less than that for the true light, and apparent deviations from Lambert's and Beer's laws will occur.

The existence of spurious light can be shown in various ways. If, with the wave-length drum set for ultraviolet or infrared light, the eye looking directly along the optical path within the instrument from the exit slit perceives colored or whitish light, spurious radiation is present. Measured deviations from Lambert's law are strongly suggestive of false light. If in the presence of a substance known to have practically complete absorption at a certain wave length a residual transmission above zero is measured, spurious light is present, and if the absorbing substance is known not to absorb at other regions, an estimate of the false light can be made. Since the stray light present at ultraviolet settings is likely to be partly of visible wave length, ultraviolet measurements are often carried out using a filter transparent to ultraviolet and highly opaque to visible (see page 1423). Even in the blue, Gibson and Balcom⁶⁸ recommended the use of a filter transmitting from 5000 to 4000 Å. for the highest accuracy with the Beckman spectrophotometer.

Wave-Length Calibration.—It is customary to check the wave-length calibration of photoelectric spectrophotometers in the visible by mapping the absorption of didymium glass. Gibson and Keegan⁷⁰ give as the average wave lengths of absorption maxima of four Corning No. 512 didymium glasses measured with 10 millimicron slits: 441.6, 475, 528.7, 585.4, 684.8, and 745 mμ, with an uncertainty of about 1 mμ. Because of the unsymmetrical form of some of the bands, some of these values change with slit

⁷⁰ K. S. Gibson and G. Keegan, *J. Optical Soc. Am.*, **31**, 462 (1941).

width, and some of the positions given are not correct for Corning 592 glasses or Jena didymium glasses.

The lines of the mercury spectrum may also be used for calibration. In instruments using a potentiometric scale for photometric indication, the potentiometer is set for a low value of transmittance, the wave-length scale set near the position of a mercury line (the slit being illuminated with a mercury arc) and the slit width adjusted to give a reading on the output meter. As the wave-length drum is turned so as to move the line across the exit slit, the meter reading will increase to a maximum; the corresponding wave-length setting should agree with the authentic wave length of the line.

Errors Due to Fluorescence.—The spectrophotometry of fluorescent solutions presents some difficulties, particularly with instruments in which the solution is placed between the exit slit of the monochromator and a photoelectric receiver. An error of 10 or 15% in a density of about 1 can occur with strongly fluorescent materials. Von Halban and Siedentopf⁶¹ point out the error caused by the entry of fluorescent light into the photocell and discuss methods of diminishing it. The absorption cell should be kept as far as possible from the photocell; and, if a lens, placed between the absorption cell and the receiver, is caused to project an image of the exit slit at a diaphragm in front of the receiver of such a size as just to transmit the image, much of the fluorescent light is blocked off.

A suitable filter, transmitting the light in the absorption band and absorbing the fluorescent light, placed in front of the photocell would diminish the error, as, for example, in the measurements of Sullivan and Norris on the photometry of riboflavin (Chapter XXII, page 1431).

The error due to fluorescence is absent in instruments in which the absorption cell is placed in front of the monochromator, except to a minor degree, at wave lengths at which the absorption and fluorescence spectra overlap.

An investigation of the errors due to fluorescence in solutions of dyes has been carried out by Dr. A. L. Geddes and Dr. S. E. Sheppard of the Research Laboratory of Eastman Kodak Company, and confirmed by Mr. E. E. Richardson of the same laboratory, to all of whom the author is indebted for discussing the problem with him.

V. MISCELLANEOUS ULTRAVIOLET SPECTRA

1. Luminescence Spectra

Certain emission spectra are of interest in organic chemistry, either for purposes of identification and estimation, or in connection with questions of structure and the like. Fluorescence, phosphorescence, chemi- and elec-

tro luminescence are examples. They all involve the determination of the position and intensity distribution of the spectra of relatively weak sources; and the spectroscopic task is similar to that of investigating a Raman spectrum (page 1371). Fluorescence and phosphorescence involve the electronic excitation of a molecule by absorption of light, and the principles of intense illumination and of avoiding spurious radiation in the emitted light apply, and, in general, the typical Raman arrangement (p. 1375) is used. Distribution of intensity can be found by the methods of photographic or photoelectric photometry. Reference may be made to the study of the fluorescence of chlorophyll by Zscheile and Harris as an example of photoelectric spectrofluorimetry.⁷¹

Phosphorescence in organic compounds is probably more common than is usually realized. Many fluorescent dyes phosphoresce if excited at low temperatures in viscous media.⁷²

Chemiluminescence, the emission of light accompanying chemical reaction, whose most common examples are the glow of the firefly and the "phosphorescence" of certain marine organisms, sometimes occurs, particularly in the oxidation of organic substances. The oxidation of pyrogallol, of organomagnesium compounds, of lophine, and of 3-aminophthalhydrazide (luminol) are well-known examples. The light yield in the last reaction has been found by Harris and Parker⁷³ to be 0.003 photon emitted per molecule of phthalhydrazide reacting. These authors describe an apparatus for concentrating the luminescence in a small region, suitable for spectrographic examination.⁷³

Electrical excitation by high-voltage discharges from an ordinary transformer causes far-reaching disruption of organic molecules, and the emission consists of that of the molecular fragments. The high-frequency Tesla discharge, however, appears to excite some molecules without decomposing them; and the spectra of aromatic compounds thus excited have been examined.⁷⁴ The spectra seem to be essentially the emission counterpart of the absorption spectra of these molecules.

2. Vacuum Ultraviolet

As has been mentioned before, the absorption of oxygen imposes a limit at a little below 2000 Å. to the spectrum that can be examined in the pres-

⁷¹ F. P. Zscheile and D. G. Harris, *J. Phys. Chem.*, **47**, 623 (1943).

⁷² See, for example, G. N. Lewis, T. T. Magel, and D. Lipkin, *J. Am. Chem. Soc.*, **64**, 1774 (1942).

⁷³ L. Harris and A. S. Parker, *J. Am. Chem. Soc.*, **57**, 1939 (1935).

⁷⁴ W. H. McVicker, J. K. Marsh, and A. W. Stewart, *J. Chem. Soc.*, **123**, 642, 817 (1923).

ence of air. The investigation of the region below 2000 Å. therefore requires the additional complexities of technique associated with the removal of oxygen. The small quartz spectrographs can be used to the limit of transparency of their optics, about 1800 Å., by passing a rapid stream of nitrogen through a side tube placed in the collimator tube near the slit and arranging a hydrogen lamp and absorption cell so as to eliminate any air path before the slit. Evacuation of the spectrograph and the use of either fluorite optics or of specially ruled gratings are required for any extensive work below 2000 Å. The important work on ethylenic compounds conducted by Carr, *et al.*,⁷⁵ at Mount Holyoke has been done by a fluorite-vacuum spectrograph and hydrogen lamp as source. The limit of transmission of fluorite is 1200 Å. or longer depending on its purity; for work at wave lengths shorter than this a grating instrument is necessary. Substances for investigation below 1200 Å. are limited to those whose vapor can be introduced directly into the spectrograph. There is a vacuum spectrograph with a grating of 21-ft. focal length at the Massachusetts Institute of Technology, but most of the grating work of interest to organic chemists has been performed with more modest instruments of one or two meters focal length. The ordinary hydrogen lamp described on page 1272 is not a satisfactory continuous source below about 1600 Å. The Lyman continuum, produced by discharging a high-capacity condenser (1 μ f. or more) through hydrogen or helium at low pressure in a capillary at the rate of about 2 flashes per sec., is the source most frequently used in the vacuum ultraviolet (see page 1277).

Schumann plates may be used in the vacuum region (page 1383). Eastman I-O plates with ultraviolet sensitization are also satisfactory.

Further information on vacuum spectroscopy must be sought in the special treatises and articles (see page 1397).

Despite its technical complications, the vacuum ultraviolet is of considerable interest to the organic chemist. In the first place, compounds containing only single-bonded carbon and hydrogen have electronic absorption spectra only in this region; the characteristic olefinic electronic absorption spectra, as in ethylene and in unconjugated polyenes, are also in this region. These are of great interest in the general problem of the correlation of spectra with structure, as well as in connection with photochemical *cis-trans* isomerization in these compounds. Of interest also is the occurrence in this region of Rydberg series of electronic transitions, analogous to the spectral series in atomic spectra, associated in molecules with transitions of non-bonding electrons.⁷⁶

⁷⁵ E. P. Carr, *et al.*, *J. Chem. Phys.*, **4**, 751, 760 (1936); **6**, 55 (1938) and later articles.

⁷⁶ See, for instance, W. C. Price, *Phys. Rev.*, **67**, 451 (1935).

VI. INFRARED SPECTROSCOPY

Until a decade or so ago, the practice of infrared spectroscopy was left to a small group of enthusiasts who spent laborious nights with temperamental instruments in the subbasements of a few university laboratories. Today it can be carried out with relative ease at any time and in almost any place under conditions in which speed in obtaining results is essential. The transition is itself largely due to the recognition that this branch of spectroscopy could be a powerful analytical tool in the solution of the traditional chemical problems of structure determination, identification and quantitative estimation; in fact, certain important problems of that sort can be solved better by this method than by any other available at present. The revolution was effected by means of instrumental improvements, particularly in the detection and automatic recording of infrared radiation, involving, for the most part the use of vacuum-tube amplifiers. The war gave a great impetus to the rapid detection of infrared radiation, and the subject is at present in a state of energetic development which promises no speedy attainment of equilibrium. This does not mean that the user of today's infrared equipment need fear rapid obsolescence; the design of spectrometers *per se* is probably rather well stabilized, and improvements in reception and recording can be readily incorporated in any spectrometer of fundamentally good design.

1. Nature of Infrared Spectra

Near-infrared spectra originate essentially in the vibrational motion of the atoms within molecules. Like all spectral processes, they are due to a transition from one energy state to another; one or more vibrational quanta may be absorbed from radiation, superposed on which, in gases, there is often a change in the rotational state of the molecule.

A nonlinear molecule of n atoms has in all $3n$ degrees of freedom,* 3 for each atom, of which 3 are associated with translational motion of the molecule as a whole and 3 with rotations of the molecule about the three principal axes. The remaining $3n - 6$ degrees of freedom are associated with vibrations of the atomic nuclei within the molecule. It behaves as if it could carry out $3n - 6$ independent modes of vibration, the *normal vibrations*, in each of which all of the atoms move in phase. Each of these modes is quantized and, if the change from one vibrational level to another in any mode causes a change in the position of the electrical center of the

* In the dynamic systems represented by a molecule regarded as a system of mass points (the atomic nuclei) subject to arbitrary small displacements in their position coordinates, the number of degrees of freedom is equal to the number of independent coordinates required to specify the configuration of the system.

molecule, it can be excited by radiation of a frequency $\bar{\nu}$ given by the universal law $h\bar{\nu} = E_2 - E_1$. The theory shows that if one quantum of vibrational energy of any normal mode is added to the molecule, the frequency of the radiation absorbed is nearly equal to the mechanical frequency of the vibration. This frequency is the greater, the smaller the

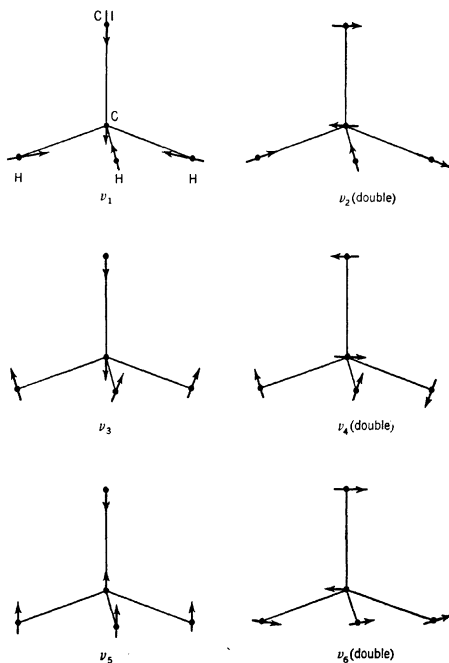


Fig. 26.—Normal vibrations of CH_3Cl .

mass of the vibrating particles and the greater the force restoring the nuclei to the equilibrium position about which they oscillate, the expression for the frequency of vibration of masses m_1 and m_2 vibrating about an equilibrium point with simple harmonic motion being:

$$\bar{\nu} = \frac{1}{2\pi} \sqrt{\frac{k}{\mu}} \quad (22)$$

where k is the restoring force per unit displacement from the equilibrium point and μ the reduced mass, $m_1 m_2 / m_1 + m_2$.

Consider, for example, methyl chloride, for which various lines of evidence indicate a tetrahedral structure as shown in figure 26. It has $3 \times 5 - 6 = 9$ normal vibrations, approximately represented in the diagrams of figure 26. In the mode ν_1 the greatest amplitude of motion is in the hydrogen atoms which oscillate about their mean positions along the direction of the valence bonds. The frequency can conveniently be termed a "C—H bond frequency" or, since one phase of the motion involves the stretching of the bond against the valence forces, it may be called a "*stretching frequency*." ν_2 is also a C—H bond frequency, in which the phase of one H atom differs by 180° from that of the others. The C and Cl nuclei are represented as vibrating in the plane of the paper, but the same frequency would be attached to a motion in which these atoms move at right angles to the plane of the paper. The actual motion of the nuclei is therefore the resultant of these equal frequencies and the frequency ν_2 is said to be "*doubly degenerate*." It is to be counted twice in the enumeration of the normal frequencies. In ν_3 the greatest amplitude of motion is still in the hydrogen atoms, which move—all in the same phase—perpendicularly to the bond; this frequency may be termed a "*bending frequency*." ν_4 is the corresponding degenerate frequency. In ν_5 and ν_6 the motion is essentially a vibration of the methyl group with respect to the Cl nucleus; in ν_5 the direction of motion is along the C—Cl bond, while in the double frequency, ν_6 , the motion is a rocking of the methyl group with respect to the Cl nucleus. The sum of the vibration forms is 9, of which 3, in this highly symmetrical molecule, are doubly degenerate, so that only 6 separate bands corresponding to the fundamental normal vibrations will be observed.

In the nondegenerate vibrations the electrical center vibrates along the C—Cl axis, and in the degenerate ones, perpendicularly to this axis. The bands associated with these motions are called *parallel* and *perpendicular*, respectively, and have spectral features which usually allow ready discrimination between the two types of motion (Fig. 27c).

A diagrammatic sketch of the absorption spectrum of methyl chloride vapor as might be obtained from a prism spectrometer is given in figure 27c. This sketch is based on the experimental work of Bennett and Meyer,⁷⁷ which was carried out with the high resolution of a grating, and includes all the bands reported by these authors. In reality each of the bands, A, B, C, etc., has a complicated rotational structure, analogous to that shown in figure 27a for HCl, but with the inadequate resolution of a prism instrument only the envelope of the bands would be revealed. A prism instrument will, however, show that two types of structure are present in the bands of a molecule like methyl chloride: (1) in which the envelope

⁷⁷ W. H. Bennett and C. F. Meyer, *Phys. Rev.*, **32**, 888 (1928).

is like that in bands *A*, *D*, and *F*, which, theory shows, is characteristic of perpendicular bands; and (2) as in *B*, *C*, *E*, and *G*, in which three more or less well-defined branches occur. The latter type of structure is characteristic of parallel bands. The central branch, weak in *B*, *C*, and *E*, and strong in *G*, is the so-called *Q* or *zero branch*. This branch is composed of rotational lines exceedingly close to one another which correspond to zero change in the rotational quantum number as the molecule is excited vibrationally. The branch on the short wave side of the *Q* branch is the *R*

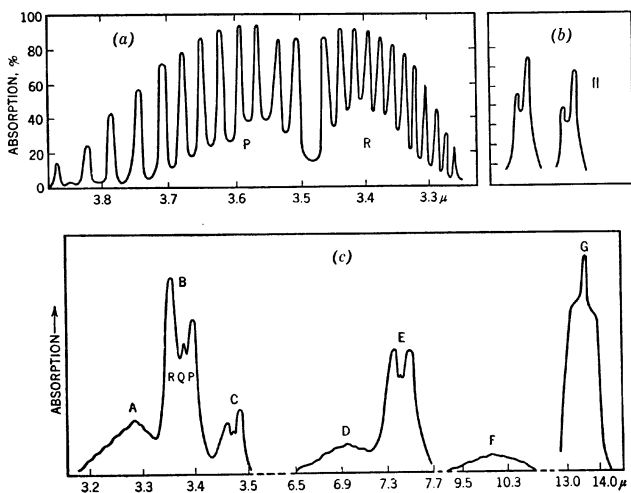


Fig. 27.—Infrared absorption spectra of gases: (a) fundamental rotation-vibration band of HCl, showing *P* and *R* branches with *Q* branch absent; (b) two of the rotational lines in this spectrum of HCl at higher resolution, showing the separate components of HCl₃₅ and HCl₃₇; (c) diagrammatic sketch of the absorption spectrum of CH₃Cl at the resolution of a good prism instrument.

or *positive branch*, in which the vibrational excitation is accompanied by an increase of rotational quantum number, and the branch on the long wave side is the *P* or *negative branch*, corresponding to vibrational excitation accompanied by a decrease in the rotational quantum number. In most diatomic molecules the *Q* branch is absent (see HCl in Fig. 27a).

It is not difficult to make a reasonable assignment of the theoretical vibrational pattern to observed bands. The two bands of longest wave length must be associated with the relatively slow vibrations of the methyl group with respect to the Cl atom, *i. e.*, *G* is $\nu_6 = 732 \text{ cm.}^{-1}$ and *F* is $\nu_8 = 1020$

cm.⁻¹. The group about $7\ \mu$ will be associated with the bending vibrations of the H atoms, which are slower than the stretching vibrations, since the restoring force for bending is less than that for stretching. E must be $\nu_3 = 1355\text{ cm.}^{-1}$ and D , $\nu_4 = 1460\text{ cm.}^{-1}$. The group of bands between 3.2 and $3.5\ \mu$, insofar as they may be fundamentals, must be associated with stretching frequencies of the C—H bonds; A is the double frequency $\nu_2 = 3047\text{ cm.}^{-1}$. It will be observed that there is one more band in the spectrum than corresponds to the 9 fundamentals (3 are double); one of the pair B and C cannot be a fundamental. The high intensity of B might suggest it as a fundamental; C would then be either the "second harmonic" of a longer band at about double its wave length, or a "combination frequency." In the former case the absorption corresponds to the excitation of two quanta in a mode of vibration, in the latter to the simultaneous excitation of two of the normal modes, *e. g.*, ν_3 and ν_4 . At present the assignment of B and C does not appear certain. It should perhaps be mentioned that the bands B and C may suffer a kind of degeneracy due to their rather close approximation to the frequencies associated with the excitation of two quanta of ν_3 and ν_4 and that ν_1 , ν_3 and ν_4 probably participate to different degrees in both bands B and C .⁷⁸

In liquids rotational structure almost invariably disappears even when it can be readily resolved in the gas. The absorption bands of liquids are often remarkably sharp: they sometimes show structure, but it is not related to the P, Q, R structure of gas bands, and apparently arises from circumstances characteristic of the condensed state. Liquid bands are usually displaced with respect to the corresponding gas bands, stretching vibrations are displaced to lower frequencies, and bending vibrations sometimes to higher frequencies.

The detailed assignment to theoretical normal vibrations of the absorption bands of molecules of some complexity and low symmetry, like most organic molecules, is very difficult; fortunately, it is unnecessary for many purposes of chemical interest. The observed bands may be divided roughly into two classes, those associated with stretching and bending "bond frequencies," and those due to motions in which larger parts of the molecule oscillate as units with respect to one another. It is an experimental fact that characteristic bands can be associated with the presence in the molecule of groups such as C—H, C \equiv N, C=C, C=O, O—H, N—H, S—H, etc. These bond frequencies, corresponding mostly to absorption bands shorter than about $8\ \mu$, although of approximately the same value in different compounds, are influenced in definite ways by the structural environment of the bond, for example, certain C—H frequencies assume

⁷⁸ K. Y. Yates and H. H. Nielsen, *Phys. Rev.*, **71**, 349 (1947).

higher values in ethylenic compounds than in saturated compounds. A knowledge of the bond frequencies of a molecule will therefore enable the organic chemist to characterize the type of molecule in more or less detail (page 1389).

The longer wave bands associated with vibrations of the parts of the molecule with respect to one another are useful in the differentiation of

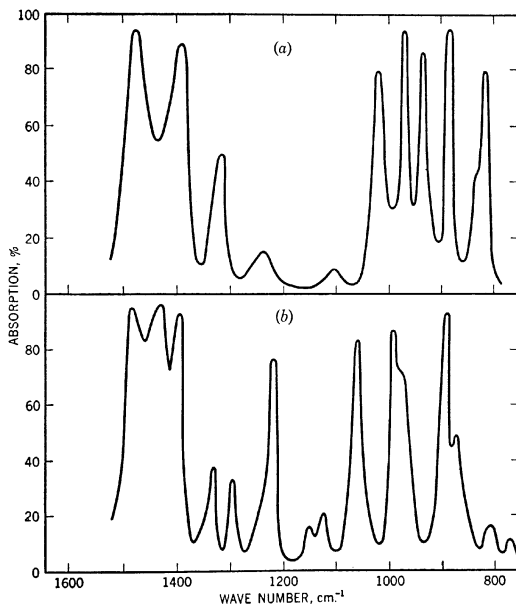


Fig. 28.—Absorption spectra of dimethyl-1,3-butadienes (after Whiffen, Torkington, and Thompson⁷⁶): (a) 1,3-dimethyl-1,3-butadiene, "key bands" at 1019, 935, and 815 cm^{-1} ; (b) 1,1-dimethyl-1,3-butadiene, "key bands" at 1218, 1061, and 995 cm^{-1} .

compounds with closely similar physical and chemical properties, such as isomeric hydrocarbons or hydrocarbons of closely similar molecular weight. Sometimes the theoretical understanding of these bands is doubtful or wanting, and the method is to make empirical comparisons between the spectra of samples of unknown composition and of pure samples. In this way analyses of such mixtures as the octane fraction of a gasoline sample,⁷⁹

⁷⁹ J. J. Heigle, M. F. Bell, and J. U. White, *Anal. Chem.*, **19**, 293 (1947).

the ortho, meta, and para cresols,⁸⁰ or the isomeric dimethylbutadienes⁸⁰ can be made quickly and accurately. Figure 28 illustrates how the infrared spectra in the region from 7 to 13 μ can be used in the qualitative and quantitative analysis of compounds as similar as 1,3-dimethyl-, and 1,1-dimethyl-1,3-butadiene.

Infrared spectroscopy has shown itself particularly well adapted to the study of certain inter- and intramolecular interactions. Inter- and intramolecular hydrogen bonding can be examined in this way. Further details on the application of infrared spectroscopy to chemical problems are given in Section IX, page 1384.

Infrared spectroscopy is probably of rather wider applicability to chemical problems than ultraviolet spectroscopy. Spectral features characteristic of molecular groupings are, it is true, found in the ultraviolet, but important classes of compounds, such as saturated hydrocarbons and hydrocarbons with unconjugated double bonds, have their ultraviolet spectra in the vacuum region, while all organic compounds absorb in the infrared. Moreover, the ultraviolet spectra of complicated molecules tend to become overlaid with continua, while in the infrared the characteristic bond vibrational bands can still be recognized in molecules as complex as those of rubber or the proteins. The possibility of photochemical changes in the ultraviolet is a factor which might sometimes point to an infrared investigation as preferable.

2. Principles of Infrared Spectroscopy

The principles of infrared spectroscopy are exactly the same as those of visible and ultraviolet spectroscopy, the differences being those of detail, imposed by the materials, sources, and receivers most suitable for use with infrared radiation. The essential equipment is a source of infrared radiation, the light from which is focused on the entrance slit of the spectrometer, within which it is collimated, dispersed, and brought to a focus at an image surface. A narrow band of the dispersed light is selected by an exit slit. The detector could be placed immediately behind the exit slit, but usually greater sensitivity is secured by focusing a reduced image of the exit slit, preferably by means of an elliptical mirror, on a small detecting element. In the spectrometer, mirrors are almost invariably used instead of lenses, silvered, or preferably aluminized or gilded by evaporation, on the front surface. Achromatism is thereby secured. These mirrors show strong astigmatism when the direction of the beams does not coincide with that of the optic axis; spherical mirrors show in addition the defects

⁸⁰ D. H. Whiffen, P. Torkington, and H. W. Thompson, *Trans. Faraday Soc.*, **41**, 200 (1945).

of spherical aberration. In instruments designed for high resolving power, parabolic mirrors are used for collimation, and astigmatism avoided by Pfund's device for working on the axis by means of an auxiliary plane mirror,⁸¹ or by so figuring the mirror that its optic axis does not coincide with its geometrical axis (a so-called off-axis mirror, as in figure 29b). Since the optical system of a spectrometer can be arranged to be symmetrical, it is possible by proper disposition of the parts to obtain at least partial compensation for the imperfections of a single mirror.

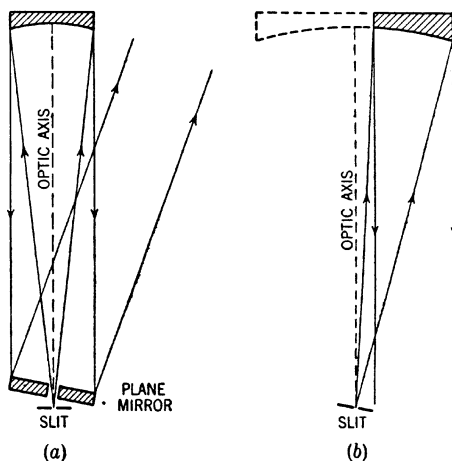


Fig. 29.—(a) Pfund's method for working "on the axis."
(b) Off-axis parabolic mirror.

A design frequently used in modern infrared spectrometers which secures maximum resolution with a minimum of optical parts is the Littrow type of instrument as illustrated in figure 30.^{82, 83} Since the radiation passes through the prism twice the dispersion is doubled and, at the same time, a single figured mirror is used. The slits *A* and *F* are bilateral and the entrance slit, *A*, is curved so as to render the image at *F* straight (page 1252). Since this correction can be perfect only at one wave length, the wave length chosen is that at which the correction is most required, where the dispersion of the prism is least. The parabolic mirror *B* is of 1-meter

⁸¹ A. H. Pfund, *J. Optical Soc. Am.*, **14**, 337 (1927).

⁸² E. D. McAlister, G. L. Matheson, and W. J. Sweeney, *Rev. Sci. Instruments*, **12**, 314 (1941).

⁸³ F. S. Brackett and E. D. McAlister, *ibid.*, **1**, 181 (1930).

focal length, cut 10° off axis. The 60° artificial rock-salt prism C is 10 cm. high and 15 cm. in face length, and is polished flat to $\frac{1}{2}$ wave length of visible light. The spectrum is swept across the exit slit, F , by rotating the plane Littrow mirror, D , by means of a mechanism, H , connected to the wave-length drum. The optical parts are mounted on a heavy, machined metal base, and provided with an air-tight cover with windows for

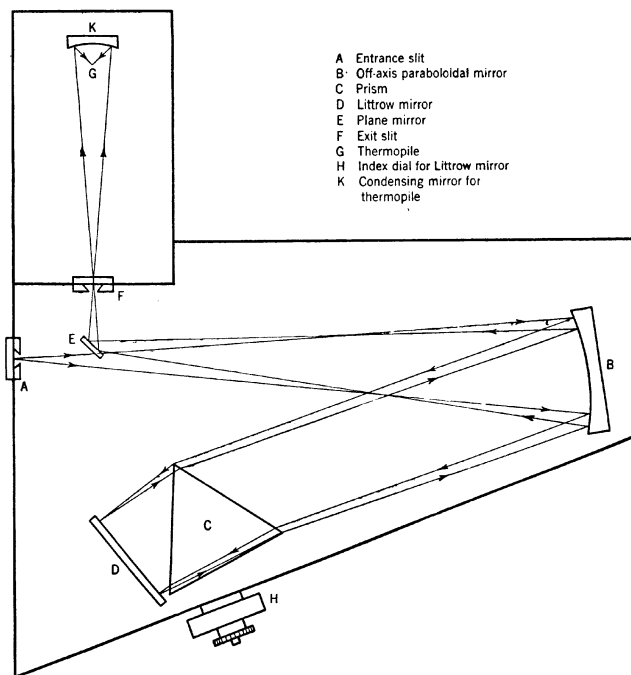


Fig. 30.—Littrow spectrometer of McAlister, Matheson, and Sweeney.⁸²

the transmission of radiation. The thermopile, G , is enclosed in a separate metal container, blackened inside with a metallic black. A diminished image of F is projected on the thermopile by the mirror K . Figure 31 is a schematic diagram of a spectrograph which combines the Littrow principle with the Wadsworth mounting.⁸⁴ The plane mirror H is placed so that

⁸⁴ R. Oetjen, Chao-Lan Kao, and H. M. Randall, *Rev. Sci. Instruments*, **13**, 516 (1942).

the axis of rotation of the prism-Wadsworth mirror system is at the intersection of the plane of the mirror and the symmetry axis of the prism. This disposition secures that, the prism having once been set at the position of minimum deviation for any one wave length, for instance, for a visible line, it is in this position for any other wave length which can traverse the optical path of the instrument on rotating the prism-mirror system.

The *Perkin-Elmer Corp.*, Glenbrook, Conn., manufactures an infrared spectrometer with an optical system similar to that of figure 30. The focal length of the collimator is 27 cm. and its aperture ratio $f/4.5$ and the prism is correspondingly smaller. The resolution with the detector and amplifier provided with the instrument is remarkable for a spectrometer of

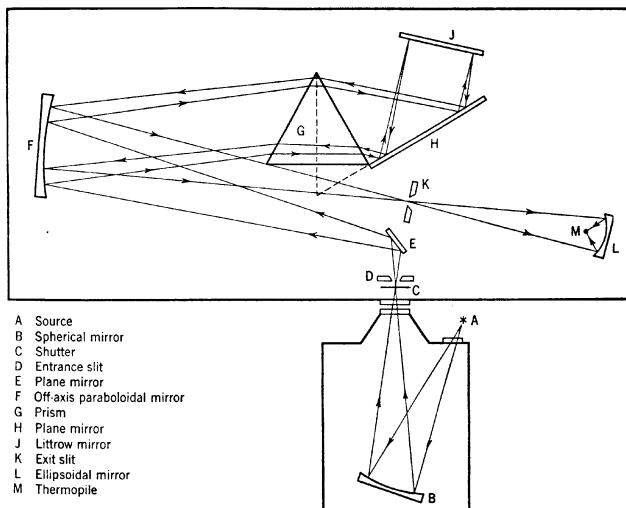


Fig. 31.—Spectrometer of Oetjen, Chao-Lan Kao, and Randall.⁸⁴

this size and, it would seem, is adequate for all normal problems likely to arise in the application of infrared methods to organic chemistry. Provision is made for the rapid interchange of prisms according to the region to be investigated.⁸⁵

Modern infrared equipment is obtainable from several manufacturers, including, in addition to *Perkin-Elmer Corp.*; *Hilger and Watts Ltd.*, London, *Baird Associates*, Cambridge, Mass., *Bausch & Lomb*, Rochester, N. Y.,

⁸⁴ R. B. Barnes, R. S. McDonald, Van Zandt Williams, and R. F. Kincaid, *J. Applied Phys.*, **16**, 77 (1945).

Gaertner Scientific Corp., Chicago Ill., *National Technical Laboratories*, South Pasadena, Calif.

3. Transmission of Materials

Table VII indicates how far from the visible the transmission extends of substances commonly used for optics and for windows of absorption cells in infrared investigations. The best material for a prism depends not only

TABLE VII
TRANSPARENCY OF PRISM MATERIALS IN INFRARED

Glass.....	to about	2 μ
Quartz.....	to about	3.5 μ
Lithium fluoride.....	to about	5.9 μ
Fluorite.....	to about	8.5 μ
Rock salt.....	to about	15 μ
Sylvine (KCl).....	to about	21 μ
Potassium bromide.....	to about	28 μ
Thallium bromide-thallium iodide ^{85a, 85b}	to about	38 μ

on its transparency, but also on its dispersion. In table VII the dispersion of any medium in the region to which it is transparent is higher than that of any medium below it. Figure 32 shows the relative values of the dispersive powers of rock salt, calcium fluoride, and lithium fluoride. The dispersion of rock salt is a minimum at about 3 μ , and is much below that of fluorite and lithium fluoride in the near infrared. Fluorite has an advantage over lithium fluoride in transparency to about 9 μ , but large pieces of the natural crystal suitable for prisms are very rare, whereas large artificial crystals of lithium fluoride are procurable. Moreover, in its region of transparency the dispersion of lithium fluoride is about twice that of fluorite. The performance of a fluorite prism spectrometer has been illustrated by Wright.⁸⁶

Quartz unfortunately has an absorption band at about 2.9 μ , beyond which the transmission rises again until the limiting absorption sets in. In general, a spectrometer provided with interchangeable lithium fluoride and rock-salt prisms⁸⁷ will be equipped for most of the problems of the organic chemist. For preliminary survey from the visible to about 15 μ , and for general work at wave lengths longer than about 6 μ , the rock-salt prism would be used, while if attention were directed, for example, toward the

^{85a} E. K. Plyler, *J. Optical Soc. Am.*, **38**, 669 (1948).

^{85b} W. L. Hyde, *J. Chem. Phys.*, **16**, 744 (1948).

⁸⁶ N. Wright, *Rev. Sci. Instruments*, **15**, 22 (1944).

⁸⁷ H. Gerchinowitz and E. B. Wilson, *J. Chem. Phys.*, **6**, 197 (1938).

C—H bands about $3.3\ \mu$ or the hydrogen-bond bands about 3 and $1.5\ \mu$, the fluorite prism would be used. Work at wave lengths longer than $15\ \mu$ requires sylvine or potassium bromide prisms.

In choosing or building a prism instrument for the infrared, attention should be paid to securing an adequately large prism. The advent of artificial crystals of materials such as rock salt, or lithium fluoride, obtainable from *The Harshaw Chemical Co.*, Cleveland, Ohio, no longer restricts one to sizes naturally available. Some modern rock-salt instruments employ prisms whose faces are of the order of 8 to $10 \times 10 \times 15\ \text{cm}$.⁸⁸

For windows, besides the substances in table VII, thin sheets of mica are useful to about $8\ \mu$, although shallow bands are present at about $2.9\ \mu$ and

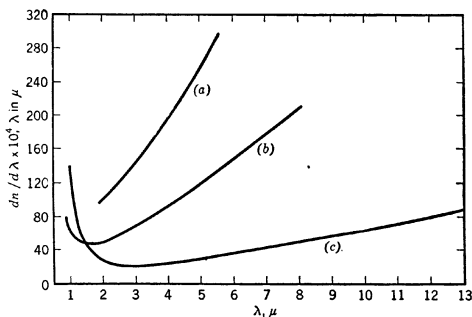


Fig. 32.—Infrared dispersion of: (a) LiF ; (b) CaF_2 ; and (c) rock salt.

between 5.5 and $7\ \mu$, according to the species. In using mica windows, care must be taken not to confuse interference bands with true absorption.

A development of the war was the production of sheets of silver chloride for use as infrared windows, making use of the long-known transparency of this substance in the infrared. Plates of silver chloride, with a transmission of about 80% to about $14\ \mu$, are available from the *Harshaw Chemical Co.* Silver chloride is plastic, and can readily be rolled out into sheets. Darkening by visible light can be delayed by dipping the chloride into sulfide solution and rolling the silver sulfide in, or by evaporating or sputtering on a coating of selenium or stibnite *in vacuo*.⁸⁹ Thallium chloride can be used in the same way.

⁸⁸ N. Wright, *Ind. Eng. Chem., Anal. Ed.*, **13**, 1 (1941). E. D. McAlister, G. L. Matheson, and W. J. Sweeny, *Rev. Sci. Instruments*, **12**, 314 (1941). W. H. Avery, *J. Optical Soc. Am.*, **31**, 633 (1941). R. A. Oetjen, Chao-Lan Kao, and H. M. Randall, *Rev. Sci. Instruments*, **13**, 515 (1942).

⁸⁹ H. C. Kremers, *J. Optical Soc. Am.*, **36**, 349 (1946).

Shutters.—The effect of spurious radiation is always more or less serious in the longer wave lengths of the infrared (see also page 1327). As is shown by figure 36 there is vastly more short-wave than long-wave energy in the normal infrared sources, and the radiation transmitted by the spectrometer at a long-wave setting is always contaminated by radiation of shorter wave length. A common method of correction is to use a shutter transparent to the short waves but opaque to the long. The short-wave impurity then acts on the receiver continuously and, when the shutter is opened, the response arises from the desired radiation. Elimination of undesired short-wave radiation by the differential scattering of films of zinc oxide or magnesium oxide smoke on rock salt or other appropriate mounts may also be useful.⁹⁰ Another method of eliminating short-wave radiation suggested by White ^{90a} is to take advantage of the selective specular reflection from a plane grating of wave lengths longer than about 1.5 times the grating spacing, while shorter wave lengths are diffracted in different directions. Replica echelettes for this purpose are supplied by *Perkin-Elmer Corp.*, Glenbrook, Conn. At wave lengths longer than 15μ , Plyler recommends a preliminary purification of the radiation from short-wave components before its entry into the spectrometer by taking advantage of the selective reflection of plates of lithium or calcium fluorides. A plane plate of the mineral placed near the source will reflect the desired radiation into the spectrometer with useful intensity and a much smaller fraction of the radiation from 2 to 14μ . The LiF plate is effective from 16 to 25μ and the CaF₂ from 20 to 25μ . In spite of these devices, a background of spurious radiation amounting to a few per cent of the observed intensity is quite likely to exist at longer wave lengths. A method of detecting and correcting for this background is described on page 1328.

4. Receivers

A. GENERAL

Great advances have been made recently in the speed and ease of detection and recording of infrared radiation, and the subject is still too much in a state of development to permit very categorical statements on "best practice." Except for certain restricted spectral regions, the most important receivers are thermopiles and bolometers, both of which indicate by being made part of an electrical system containing some kind of meter. The desiderata are easily stated as follows: high effective sensitivity to radiation and high speed of response with freedom from extraneous dis-

⁹⁰ A. H. Pfund, *Phys. Rev.*, **36**, 71 (1930); *J. Optical Soc. Am.*, **24**, 143 (1934).

^{90a} J. U. White, *J. Optical Soc. Am.*, **37**, 713 (1947).

⁹¹ E. K. Plyler, *Phys. Rev.*, **72**, 165 (1947).

turbance. Thermopiles operate by means of the potential change which is developed when one or more junctions between dissimilar metallic conductors change in temperature as the result of exposure to radiation. In a compensated thermopile two junctions are used, as nearly identical as possible, connected in such a way that the thermal e. m. f.'s are in opposition. One is exposed to the radiation, the other, in the same housing, is shielded. Effects of temperature changes in the general surroundings, which otherwise lead to drift in the zero position of the indicator, are therefore largely eliminated and the response when the active junction is exposed to radiation is a true measure of the radiation.

The sensitivity, Q , of an uncompensated thermopile is given by an expression of the form:

$$Q = \frac{\text{number of junctions} \times \text{thermoelectric power}}{(\text{total circuit resistance})^{1/2}} \times \frac{1}{\text{losses}} \quad (23)$$

where the losses are composed of radiation loss from the thermopile plus gas conduction loss plus conduction loss along the metallic components plus the Peltier loss across the junction.⁹² The Peltier loss is normally not an important factor in thermopile design. To minimize radiation losses the area and radiative power are kept low, the gas conduction loss is reduced by evacuation of the housing, and the metallic conduction losses by choice of small pieces of metals with as low as possible a ratio of thermal to electrical conductivity (Wiedemann-Franz constant). The thermoelectric power is of course chosen as high as possible, consistent with appropriate values of the other constants. For spectroscopic purposes the best compromise among the conflicting factors is to use a single junction for the reception of the radiation with a small receiver about 0.2 mm. in breadth and 5 mm. in length; a favorable junction is that between bismuth and an alloy of bismuth with 5% tin. For high speed of response the heat capacity of the junction and receiver should be kept at a minimum. This is often accomplished by forming the junctions from very thin evaporated films, but recently it has been shown that high speed of response can be secured from wire-constructed elements if the very thin wires are sufficiently short.⁹³

B. THERMOPILE SYSTEMS WITHOUT ELECTRONIC AMPLIFICATION

It may facilitate an understanding of the principles involved in the use of thermopiles to start with a description of the simplest kind of thermopile-galvanometer system. A method of constructing wire thermopiles is fully

⁹² C. H. Cartwright and J. Strong, *Procedures in Experimental Physics*. Prentice-Hall, New York, 1939, p. 333.

⁹³ D. F. Hornig and B. J. O'Keefe, *Rev. Sci. Instruments*, **18**, 474 (1947).

described by Cartwright and Strong.⁹² The junction is bismuth against bismuth-tin in the form of wires about $24\ \mu$ and $31\ \mu$ in diameter, respectively, and about 3 mm. long. The radiation is received by a blackened receiver over the junction, a few mm. long and a fraction of a mm. wide; a reduced image of the exit slit is formed so as just to fill the receiver. To the housing of the junctions (two are used for compensation) is attached a side chamber containing charcoal which, by immersion in liquid air, maintains a vacuum. The thermopile assembly is sealed off under vacuum with the charcoal vessel heated, and occasional reevacuations may be necessary. In testing this system for leaks, a high-frequency "leak tester" must not be used as the element may be destroyed. A good single-junction thermopile of this type will develop about 10^{-6} volt for a flux of incident radiation of 10^{-6} watt per mm.². Several seconds are required for the attainment of equilibrium between the thermopile and the radiation.

In the most direct method of measuring the thermal e. m. f.'s developed by the thermopile, a low-resistance galvanometer of high voltage sensitivity is used. For maximum sensitivity of the thermopile-galvanometer system, the resistance of the pile and of the galvanometer should be about the same. The Leeds and Northrup Type H.S. instrument, with a resistance of about 10 ohms and a sensitivity of about 0.05 microvolt per mm. deflection at 1 meter and a critically damped period of about 7 seconds is representative of the type.

The sensitivity of such a system can be increased by means of a *thermoelectric* or *photoelectric relay*. In the Moll and Burger Thermo-relay, made by Kipp & Zonen, Delft, Holland, a beam of light reflected from the mirror of the primary galvanometer is made to fall on a bimetallic strip connected to a second galvanometer. With no radiation incident on the thermopile, the beam from the primary galvanometer is made to fall centrally on the bimetallic strip so that no e. m. f. is developed, and the secondary galvanometer, connected to the strip, registers zero. As the mirror of the primary galvanometer is deflected in response to the reception of radiation by the thermopile, the light beam heats the two components of the strip unequally, and a thermal e. m. f. is developed which may produce a deflection in the secondary galvanometer many times greater than the original deflection in the primary.

The same kind of amplification can be effected by using photoelectric cells. Particularly simple is a system using a barrier layer cell divided in two, the two halves being connected in opposition (Fig. 33). Such cells are commercially available. An image of an illuminated slit is reflected by the mirror of the primary galvanometer so as to illuminate equally the two halves of the photocell on either side of the dividing line. A small deflection illuminates one half more than the other and produces a photocur-

rent measured by a second galvanometer. It is convenient to mount the photocell on a platform with a screw-controlled motion perpendicular to the beam, for ease in setting the zero position. Systems with photoelectric relay amplification have been successfully used in automatic recording spectrometers.⁹⁴ Such amplification may be carried out with two objects: (1) to gain the highest sensitivity attainable, in which case a sensitive and correspondingly slow galvanometer would be used as primary; or (2) to attain the sensitivity normally attainable by a single highly sensitive instrument by means of galvanometers of greater speed of response,

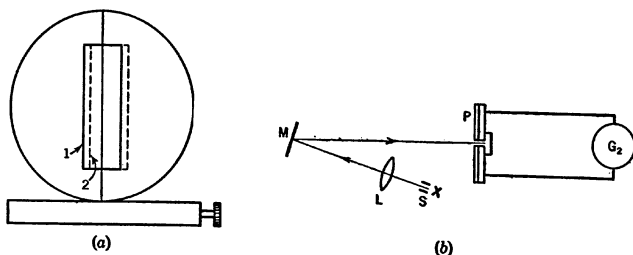


Fig. 33.—Relay amplification by means of divided photovoltaic cell: (a) elevation of photocell, showing the positions of the light beam reflected from the primary galvanometer at zero (position 1), and at a deflection (position 2); (b) optical and electrical arrangement, S, illuminated slit, projected by lens, L, as an enlarged image at photocell, P, after reflection at mirror, M, of primary galvanometer (G_2 is the secondary galvanometer which should be of relatively low resistance and moderate sensitivity).

and therefore lower sensitivity. Taut-suspension galvanometers of moderate sensitivity, considerable stability, and of a period of about two seconds are convenient in this application. The Moll Galvanometer, made by Kipp & Zonen, is an example. By using two of these in a relay system a sensitivity greater than that of a long-period, highly sensitive galvanometer alone can be achieved with a very considerable gain in speed. Amplification by this means is limited by the steadiness of the primary galvanometer. Great care must therefore be taken to eliminate mechanical vibration and electrical and electromagnetic disturbances. If these are absent, the ultimate limit is set by the Brownian motion of the galvanometer mirror (see page 1354).

If a basement room is used, vibration can be reduced by mounting galvanometers and amplifying systems on concrete piers brought up from the foundations of the building through the floor. The author's experience in

⁹⁴ For example, N. Wright, *Ind. Eng. Chem., Anal. Ed.*, **13**, 1 (1941).

the sub-basement of a large and busy city building which happened to be founded on rock was that adequate steadiness of the galvanometer was secured by placing it on a lead or heavy iron platform resting on a fine-texture sponge rubber mat on a heavy wooden tripod stand. The weight on the stand must be large enough to give high inertia but not so heavy as to keep the rubber from being springy; the weight required is best found by trial and error. The total thickness of the rubber should not be much over 1.5 inches, or sideways will set in.

In upper rooms, some kind of special vibrationless galvanometer support will probably be necessary. The taped spring support described by Johnsrud⁶⁶ has been found completely satisfactory in arduous vibrational conditions.

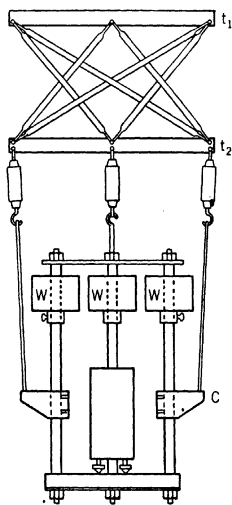


Fig. 34.—Vibrationless suspension.

In figure 34, t_1 and t_2 are two triangles of iron strips. Hooked into them at the corners are S-hooks, connected to coil springs, as common screendoor springs. The springs are 15 inches long before they are tensed and 18 to 19 inches after. Suspended from the bottom triangle by means of turnbuckles and extension rods or wires is a frame consisting of three vertical rods held firmly at the ends in round plates. Adjustable in height on these rods are three cans, W , which can be weighted with lead shot. The position and masses of W are determined by the condition that the center of oscillation of the frame and galvanometer coincide with the point of support of the galvanometer coil. The three clamps, C , are at such a height on the rods that the plane of the three points of support of the frame contains the point of suspension

of the coil. A lead slab is placed on the lower plate of the frame. The springs are tightly wound with friction tape over the entire length, each turn overlapping the previous one by about half the width. This is done after the suspension is hung but before the galvanometer is put in place. The top triangle, t_1 , must be firmly clamped so as not to turn or tilt with respect to the building. If necessary, other stories of springs can be added.

A simpler arrangement, consisting of a heavy frame similar to that of figure 34, suspended from the ceiling by a long single strand of piano

⁶⁶ A. L. Johnsrud, *J. Optical Soc. Am.*, 10, 609 (1925).

wire, with a vane immersed in oil below the floor of the support to dampen torsional oscillations, is sometimes effective; and a simple suspension that can be set on the bench has been described by Müller.⁹⁶

Electrical connections to galvanometers should be intertwined insulated wires fastened as firmly as possible to some support. A loose wire moving in the earth's magnetic field can produce erratic fluctuations. If electrical disturbances from high-tension apparatus in the neighborhood are probable, the whole thermopile-galvanometer system should be electrically shielded. The braided metal shields common in radio apparatus are useful for the leads, and the galvanometer is placed inside a grounded metal cover. Some infrared workers, whose laboratories are near rooms in which high-tension spark sources are in use, line their rooms with sheet metal.

C. THERMOPILE SYSTEMS WITH ELECTRONIC AMPLIFICATION

The most important recent advances in infrared technique have centered around the development of fast receivers, most of whose response to infrared radiation is completed in less than a second, and in the electronic amplification of the initial small response so that the final indication is made by means of relatively rugged instruments such as self-recording milliammeters. The difficulties of d.-c. amplification of very small voltages are avoided by using a.-c. amplifiers with some means of converting the initial d.-c. response of the receiver into an alternating voltage. In one method, developed in the research laboratories of *General Motors Corp.*,⁹⁷ the current from the thermopile is fed into a mechanical interrupter or "breaker" and the interrupted voltage applied to the input of a reactance-coupled amplifier through a step-up transformer. The output circuit contains a breaker driven synchronously with the input breaker, and the rectified output signal can be recorded by means of a recording milliammeter. An important function of the output breaker is to allow only signals of the same frequency as the input interrupter to produce a d.-c. component in the output circuit; induced voltages and amplifier "noises" of other than breaker frequency will not be rectified and therefore not recorded by the meter.

The success of this method is largely dependent on the quality of the interrupters. Commutators and commercial vibrators were found unsatisfactory, and specially made interrupters using a nonsliding contact, free from chatter, were made. Care must be taken to eliminate all variation of thermal voltages in the input circuit in order to minimize drift. The

⁹⁶ R. Müller, *Ann. Physik*, **1**, 613 (1929). J. Strong *et al.*, *Procedures in Experimental Physics*, Prentice-Hall, New York, 1939, pp. 328, 591.

⁹⁷ M. D. Liston, C. E. Quinn, W. E. Sargeant, and G. G. Scott, *Rev. Sci. Instruments*, **17**, 194 (1946).

entire input circuit is shielded from changes in the ambient temperature by being housed in a cork-lined box. In order to reduce thermoelectric disturbance in the input, metallic joints are avoided whenever possible and, where necessary, are made with a special solder (70% cadmium, 30% tin) for use against hard-drawn electrical copper, with a low thermoelectric potential against copper. For details, the original article should be consulted. The erratic fluctuations in this amplifier with an input resistance of 5 ohms and an Esterline-Angus recording milliammeter giving full-scale deflection for 5 ma. were not greater than 1.5×10^{-9} volt, and the amplifier drift under laboratory conditions was less than 5×10^{-9} volt in 8 hours.

It will be noticed that this amplifier in itself does not require a fast receiver. The amplifier would be quite satisfactory for use with the sensitive thin-wire thermocouples already described. Thermopile drift is not eliminated by this amplifier, so that a compensated thermopile should be used.

If a thermopile of sufficiently fast response can be used, it is possible to interrupt the light beam periodically before it enters the spectrometer by means of a rotating shutter, and so to obtain an alternating output from the thermopile. This can then be amplified by means of an a.-c. amplifier tuned to the frequency of interruption of the light beam. Disturbances of other frequencies, such as thermopile drift, erratic electrical and electromagnetic disturbances, mechanical vibration, and stray radiation not modulated at the operating frequency are therefore not recorded and an appreciable gain in stability is secured.

There is a considerable literature on the production of thermopiles intended for use with a.-c. amplifiers; Harris and his coworkers have been most active in this field.⁹⁸ The prime necessity is to reduce the heat capacity and thermal conduction of the thermopile to very low values by the use of thin films of materials with high thermoelectric potentials and low ratios of thermal to electrical conductivity. The design and construction of such rapid-response thermopiles for use in infrared spectrometers has been considered in detail by Roess and Dacus.⁹⁹ As for the wire thermopile, a single-junction instrument has the maximum usable sensitivity for infrared spectroscopy. Films of bismuth and antimony 600 to 1000 Å. thick are condensed by evaporation *in vacuo* on Formvar¹⁰⁰ films 400 to 500 Å. thick, the metal films overlapping slightly to form the junction; the Formvar film is carried by a glass support. The completed thermopile

⁹⁸ L. Harris, *Phys. Rev.*, **45**, 635 (1934). L. Harris and E. A. Johnson, *Rev. Sci. Instruments*, **4**, 454 (1933). L. Harris and A. C. Scholp, *J. Optical Soc. Am.*, **30**, 519 (1940).

⁹⁹ L. C. Roess and E. N. Dacus, *Rev. Sci. Instruments*, **16**, 164 (1945).

¹⁰⁰ A Polyvinyl formal resin made by the *Shawinigan Products Corp.*

has a resistance of 20 to 50 ohms, a direct current sensitivity of 6 to 7 $\mu\text{v.}$ for a radiation flux of 10^{-6} watt per mm.^2 and an output to radiation interrupted at 7 cycles per second of not less than half the d.-c. value. The thermopile is used in conjunction with an amplifier¹⁰¹ sharply tuned to the frequency of modulation of the radiation beam. The breaker type of amplifier previously mentioned (which operates at a frequency of about 75 cycles per second) could also be used in conjunction with an a.-c. thermopile operating at about 5 cycles per second.

D. BOLOMETERS

The bolometer is essentially a resistance thermometer provided with a blackened receiver for the absorption of radiation. It is made of a thin strip of conducting material with a high temperature coefficient of resistance, which is arranged to form one arm of a Wheatstone bridge. Usually an identical strip protected from the radiation forms another arm of the bridge and acts as a compensator toward changes in the ambient temperature, while two equal resistances with negligible temperature coefficient complete the bridge. There is an optimum working current, usually of several hundredths of an ampere, which must be supplied by an external source. The unbalance in the previously balanced bridge effected by the radiation to be measured can be directly indicated by a sensitive galvanometer; or the indication of a less sensitive high-speed galvanometer, such as the micro Moll galvanometer of 0.2 second period, can be amplified by a photoelectric relay (page 1346),¹⁰² or vacuum-tube amplification can be employed. Bolometers have recently returned to favor because of the relative ease of obtaining quick response. They are therefore suitable for use with interrupted beams and tuned a.-c. amplifiers, and have been used in spectrometers in which the spectrum out to $15\ \mu$ is scanned in one minute and displayed on the screen of an oscilloscope.¹⁰²

The thin metallic films may be made either by rolling foil or by evaporation. Baker and Robb obtained strips of platinum foil, $1\ \mu$ thick, 0.03 mm. wide, and 5 mm. long, by rolling out platinum between silver to a thickness of 0.001 inch. Strips cut from the composite foil are soldered in place, and the silver is then removed by electrolysis in concentrated potassium cyanide solution followed by a quick dip in nitric acid solution. The strips are then blackened by evaporating a very thin layer of antimony black. For this bolometer the time required for the radiation to change the resistance to 99% of the final value was 0.089 second, the optimum working current was 0.0346 amp., and the minimum radiation measurable with an uncertainty of 1% due to the inherent unsteadiness of Brownian motion

¹⁰¹ L. C. Roess, *Rev. Sci. Instruments*, **16**, 172 (1945).

¹⁰² E. B. Baker and C. D. Robb, *Rev. Sci. Instruments*, **14**, 356 (1943).

effects was 6×10^{-8} watt (see page 1354). The construction of bolometers consisting of evaporated films of gold 0.05 to 0.1 μ thick and capable of being used satisfactorily with radiation modulated at 30 cycles per second is described by Aiken, Carter, and Phillips.¹⁰³

A development which promises to be of importance in infrared spectroscopy is the "Thermistor" bolometer.¹⁰⁴ Thermistors are resistors made of semiconducting mixtures of metallic oxides such as Mn_2O_3 , NiO , with a very high temperature coefficient of resistance.¹⁰⁵ Like all semiconductors they decrease in resistance with increasing temperature, and their negative temperature coefficient of resistance at 25° C. is much higher than the positive temperature coefficient of metals. In the form of thin flakes about 10 μ thick they form sensitive bolometers with thermal time constants of 0.003 to 0.005 second. They are normally operated at atmospheric pressure. An amplifier is commercially available which is suitable for use with these bolometers with a narrow pass band, 2 cycles wide, centered at 15 cycles per second. If the incident radiation is modulated at 15 cycles per second, the combination shows the insensitivity to temperature variations and erratic disturbances, characteristic of tuned systems.

What will probably turn out to be an important development in infrared spectroscopy was initiated by Baker and Robb¹⁰² in their introduction of oscillographic display of the infrared spectrum as received by a fast bolometer. Thermistors would seem particularly suitable for this purpose and Daly and Sutherland^{106, 107} have photographed infrared spectra in this way as have H. W. Thompson and coworkers.^{107a} A range of 2.5 to 3.5 μ between 1 and 16 μ can be scanned in 14 seconds at a resolving power sufficiently good for most work on complex molecules. Figure 35 reproduces the oscillographic tracings obtained for 2,2,4-trimethylpentane and 2,2,5-trimethylpentane,¹⁰⁷ and incidentally shows the power of the infrared method for the identification of certain classes of closely related isomers. To quote the authors: "When one considers that these two plots were obtained in a time of approximately 30 seconds, and that there is every likelihood that this time can be reduced by a factor of 5 in the very near future, the potentialities of this instrument as a tool for chemical research and analysis need no further emphasis."

Near the superconducting transition point the temperature coefficient of resistance of metals becomes enormous, and by using thin strips of columbium nitride, CbN , in this condition, Andrews and his coworkers have produced probably the most sensitive radiation meter of the nonselec-

¹⁰³ C. B. Aiken, W. H. Carter, and F. S. Phillips, *Rev. Sci. Instruments*, **17**, 377 (1946).

¹⁰⁴ Obtainable from *Graybar Electric Co., Inc.*, 180 Varick St., New York 14, N. Y.

¹⁰⁶ J. A. Becker, C. B. Green, and G. L. Pearson, *Bell System Tech. J.*, **26**, 170 (1947).

¹⁰⁷ E. F. Daly and G. B. M. Sutherland, *Nature*, **157**, 546 (1946).

tive kind yet found.^{108,108a} The response is very rapid and 6×10^{-8} watt is equivalent to one hundred times the inherent unsteadiness of the bolometer-amplifier system. Liquid-hydrogen temperatures are unfortunately necessary, so that the use of this type of bolometer must remain restricted until either an important demand arises for liquid hydrogen or until a system with a transition point accessible to the common refrigerants is found.

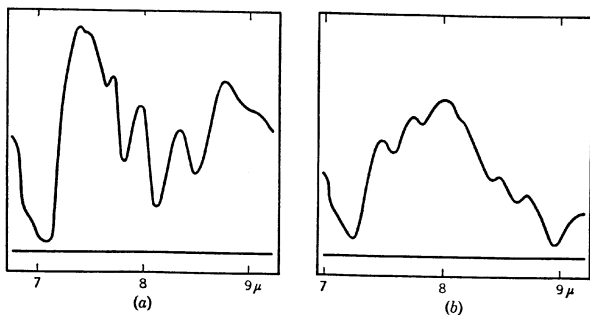


Fig. 35.—Oscillographic presentation of infrared spectra:
(a) 2,2,4-trimethylpentane; (b) 2,3,4-trimethylpentane.¹⁰⁷

E. PNEUMATIC DETECTOR

An entirely different kind of detector, resembling the thermopile and bolometer in being nonselective, has been described by Golay.¹⁰⁹ The receiver is a small, gas-filled chamber in the center of which is a thin, blackened membrane. On exposure to radiation the gas expands and the effect is communicated through a narrow channel to a light, flexible mirror, which acts as the detecting element. This mirror is illuminated with visible light from the back of the cell, and is part of an optical system consisting of a lamp, projecting lenses, detecting mirror, and photoelectric cell which causes the photocell to be exposed to more or less light as the mirror is distorted by the pressure pulse produced in the receiver. The photocell is connected to an electronic amplifier. The incident infrared light is modulated in the usual way by means of a rotating shutter in front

¹⁰⁷ E. F. Daly and G. B. M. Sutherland, *Proc. Phys. Soc. London*, **59**, 77 (1947).

^{107a} J. King, R. B. Temple, and H. W. Thompson, *Nature*, **158**, 196 (1947).

¹⁰⁸ D. H. Andrews, R. M. Milton, and W. De Sorbo, *J. Optical Soc. Am.*, **36**, 353 (1946).

^{108a} N. Fuson, *ibid.*, **38**, 845 (1948).

¹⁰⁹ M. J. E. Golay, *Rev. Sci. Instruments*, **18**, 357 (1947).

of the entrance slit, and the a.-c. output of the amplifier is rectified in synchronism so as to yield a direct current proportional to the intensity of the radiation reaching the receiving chamber. In a typical receiver the time constant was about 0.003 second and the incident flux equivalent to one hundred times the inherent fluctuations was 1.4×10^{-7} watt. A gain in speed at the expense of sensitivity was secured by using helium instead of air, when a time constant of 0.0006 second was obtained.

F. SENSITIVITY OF RECEIVERS

In any system consisting of a thermopile or bolometer and its indicating apparatus there is an inherent upper limit of sensitivity beyond which no further useful sensitivity of the system can be realized. This limit exists in spite of the complete elimination of sources of avoidable disturbances, such as mechanical vibration, external electrical, magnetic, and electromagnetic disturbances, avoidable variations in the supply voltages in amplifiers, and so on. The limit is set by Brownian motion fluctuations. In the thermopile there are Brownian fluctuations of temperature, the mirror of a galvanometer is subject to Brownian oscillations by virtue of its thermal energy of *ca.* kT , and all resistances in the circuit show current fluctuations due to the Brownian motion of electrons. As a result, the mirror of the galvanometer in a typical thermopile circuit, in the absence of all controllable disturbances, carries out erratic fluctuations equivalent to the indications of a voltage equal to $(\pi kTR/P)^{1/2}$ where k is the Boltzmann constant, R the total circuit resistance, P the period of the galvanometer, and T the absolute temperature. At 20° C. the expression reduces to $1.13 \times 10^{-10} (R/P)^{1/2}$, and amounts to about 1.6×10^{-10} v. for a galvanometer of 10-second period in a circuit with a total resistance of 20 ohms. Sensitive galvanometer-thermopile systems therefore cannot be usefully amplified more than a hundred times by such devices as photoelectric relays. In a similar way the useful sensitivity of vacuum-tube amplifiers is limited by the Brownian motion of the electrons in the resistances. In any case, whether they are determined solely by the uncontrollable effects intrinsic in the atomic nature of matter, or whether they originate in disturbances that can be removed in principle, but whose elimination may be very difficult in practice, the fluctuations of zero in any radiation-measuring system determine the useful sensitivity. In amplifiers at audio frequencies the erratic fluctuations are heard as a background jumble of noise, and the term is convenient to describe the background of erratic fluctuations in any instrument. The noise determines the smallest amount of energy detectable by the system, and the accuracy with which larger signals may be read.

In table VIII are collected estimates of the amount of radiation which

produces a signal equal to the noise for a number of detecting systems described in the literature. For a single reading to be made with an accuracy of about 1% the signal should be 100 times the noise value. In the table, t is the time required for the detector to complete most of its response, and N is the energy equivalent to noise. The receivers are all of a size and shape suitable for spectrometry and the area in most cases is about 1 mm.².

TABLE VIII
NOISE LEVELS FOR DETECTORS

Receiver	t , sec.	Indicator	N , watt
Wire thermocouple	2 or more	Sensitive galvanometer with relay amplification, period 10 sec., and total resistance 20 ohms (Brownian limit)	2×10^{-10}
		Galvanometer with relay amplification, period 2 sec., total resistance 100 ohms (Brownian limit)	8×10^{-10}
		Breaker-type vacuum-tube amplifier ^a	1.5×10^{-10}
Fast thermocouple	0.14	Vacuum-tube amplifier, 1-5 cycles/sec. ^b	3×10^{-10}
Platinum strip bolometer	0.089	Vacuum-tube amplifier ^c	6×10^{-10}
Supraconducting bolometer	0.0005 (primary)	Vacuum-tube amplifier ^d	6×10^{-10}
	0.02 (secondary)		
Thermistor bolometer	0.005	Vacuum-tube amplifier ^e (overall time constant, 1.5 sec.)	1×10^{-10}
Pneumatic receiver	0.003	Photoelectric cell + vacuum-tube ^f relay amplification	1.4×10^{-9}
PbS photocell at 2.5 μ , at -80°	Fast	Vacuum-tube amplifier ^{g, h}	3.3×10^{-12}

^a Liston, Quinn, Sargeant, and Scott, *Rev. Sci. Instruments*, **17**, 194 (1946).

^b Roess, *ibid.*, **16**, 172 (1945). ^c Baker and Robb, *ibid.*, **14**, 356 (1943).

^d Andrews, Milton, and DeSorbo, *J. Optical Soc. Am.*, **36**, 353 (1946).

^e Becker and Moore, *ibid.*, **36**, 354 (1946).

^f Golay, *Rev. Sci. Instruments*, **18**, 357 (1947).

^g Cashman, *J. Optical Soc. Am.*, **36**, 356 (1946).

^h Oxley, *ibid.*, **36**, 356 (1946).

Note: For a critical discussion of the performance of radiation detectors, see R. C. Jones, *ibid.*, **37**, 879 (1947); **39**, 327, 344 (1949).

G. OTHER RECEIVERS FOR THE INFRARED

Appropriately sensitized *photographic plates* can be used out to about 1.2 μ . The instruments and the technique are the same as in photographic spectrophotometry and spectroscopy in the visible and ultraviolet.

Photocells.—Like the photographic plate, the well-known photoelectric cells and photovoltaic cells are limited to application in the near infrared. During the war there was considerable development of exceptionally sen-

sitive lead sulfide photoconductive cells with a threshold at $3.6\ \mu$, maximum sensitivity at $2.5\ \mu$, and sensitivities of 20% of the maximum at $3.3\ \mu$ and at $4000\ \text{\AA}$.^{110, 111} The sensitivity at the temperature of dry ice is about 100 times that at room temperature. The cells can be operated without serious loss in sensitivity at frequencies of 90 cycles per second, and at the wave length of maximum sensitivity their sensitivity is such that an energy flux of about 3.3×10^{-10} watt per mm^2 produces a signal 100 times greater than the noise of the cell-amplifier system, a sensitivity, at that wave length, of the order of 100 times that of the thermopile systems in common use. The wave-length range in the infrared is too restricted to make this cell useful for general analytical or structural work, but it appears valuable for special problems in the region shorter than $3.4\ \mu$. Studies of C—H vibrations and other vibrations involving light atoms and hydrogen, hydrogen-bond effects, and the study of overtones and combination frequencies could be carried out by means of this receiver. With the use of a cell of this type, bands in the water vapor spectrum as close as $0.14\ \text{cm}^{-1}$ at $2.5\ \mu$ have recently been resolved.¹¹²

Selective Evaporation.—Only brief mention can be made of detection by selective evaporation of volatile substances, which method has not been widely used but which has certain possibilities of development. A plate carrying a film of volatile material is placed at the image plane of the spectrometer, and selective evaporation takes place at the spots where the radiation is most intense. The film, differentiated according to the intensity of the spectrum incident on its various parts, can be photographed and a permanent record made. This is indeed the method by which Sir John Herschel in 1840 demonstrated the existence of absorption bands in the infrared spectrum of the sun, by the selective evaporation of alcohol. At the hands of Czerney and his coworkers, the method, by which the image produced in films of volatile materials such as naphthalene is then photographed, showed considerable promise of successful development.¹¹³

Phosphorescence Methods.—A method of limited possibilities depends on the effect of near-infrared radiation on phosphorescence. Near-infrared light may quench the phosphorescence produced by visible or ultraviolet light in materials like calcium sulfide phosphors, or it may render an already excited phosphor more luminescent. Both phenomena can be used to detect infrared spectra: in the first method¹¹⁴ the infrared wave lengths

¹¹⁰ C. L. Oxley, *J. Optical Soc. Am.*, **36**, 356 (1946).

¹¹¹ R. J. Cashman, *ibid.*, **36**, 356 (1946).

¹¹² G. B. B. M. Sutherland, D. E. Blackwell, and F. B. Fellgett, *Nature*, **158**, 873 (1946).

¹¹³ Monch and Willenberg, *Z. Physik*, **108**, 85 (1937).

¹¹⁴ For example, Bergmann, *Z. wiss. Phot.*, **6**, 113 (1908).

of high intensity appear as dark bands against a more brightly luminescent background, and in the other,¹¹⁵ as bright bands against a darker background. The luminescent plate with its impressed spectrum can be photographed by suitably sensitized plates.

5. Sources

From the visible to about $2\ \mu$, a 500-watt tungsten-filament projection amp is a convenient source. The Nernst Glower¹¹⁶ is a very convenient infrared source. This is a cylindrical filament, 1 to 2 cm. long and about 0.5 mm. in diameter, composed of a mixture of zirconium and yttrium oxides, which can be operated in air from a 110-v. supply, drawing a current of 0.8 to 1 amp. It has a negative temperature coefficient of resistance, and must be stabilized with a ballast resistance, as a tungsten-filament lamp of suitable resistance, or, very conveniently, by the type of current control consisting of an iron filament mounted in hydrogen, obtainable from radio supply stores (baretters). For constancy of output the glower and ballast resistance should be operated from a constant-voltage transformer.

The life of the commercial Nernst Glower is limited by failure of the thin platinum wires leading from the ends of the filament to the terminals for connection in the circuit. Even before fusion of the platinum, fluctuations of intensity may be caused by imperfections of this contact. This connection can be strengthened¹¹⁷ by winding 6 or 8 inches of No. 30 platinum wire into a ball at each end of the Nernst filament and carefully melting the wire into a globule or ring by means of an oxygen-gas flame. A short piece of No. 23 platinum wire is then welded to the ball by holding the two in contact in an oxygen-gas flame which is not too hot. A length of No. 30 platinum wire is spot-welded or hammer-welded to the thicker platinum wire, and serves for attachment to the terminals at which the power is brought to the glower. The flexibility of this thin wire prevents the introduction of strains in the filament during operation. The Nernst glower is a conveniently small source which does not unduly heat the room or the adjacent apparatus. Normally a water-cooled jacket is unnecessary with this source.

Another source frequently used is the *Globar*, a rod of silicon carbide made by *Carborundum Corporation*, Niagara Falls, N. Y., for use as heating elements in furnaces. They are procurable in a variety of sizes of which the

¹¹⁵ F. W. Paul, *J. Optical Soc. Am.*, **36**, 352 (1946).

¹¹⁶ Obtainable from *Gaertner Scientific Corp.*, Chicago, Ill., *Stupakoff Laboratories*, 6627 Hamilton Ave., Pittsburgh, Pa., and *National Technical Laboratories*, South Pasadena, Calif.

¹¹⁷ E. S. Ebers and H. H. Nielsen, *Rev. Sci. Instruments*, **11**, 29 (1940).

smaller ones, 5–10 cm. long and about 6 mm. in diameter, are most convenient as sources. The rod may be mounted between aluminum cup electrodes (obtainable from the maker) held together by springs; a number of alternative mountings are described.¹¹⁸ To prevent changes in room temperature the Globar should be operated surrounded by a water-cooled jacket. In the region longer than $10\ \mu$ the Globar emits relatively more energy than the Nernst Glower.

Smith¹¹⁹ describes the use of a carbon rod electrically heated *in vacuo* to 1800°C . with 600 watts and a lifetime of 100 hours; the emission is about equal to that of the Globar from $1\text{--}10\ \mu$ and higher at longer wave lengths.

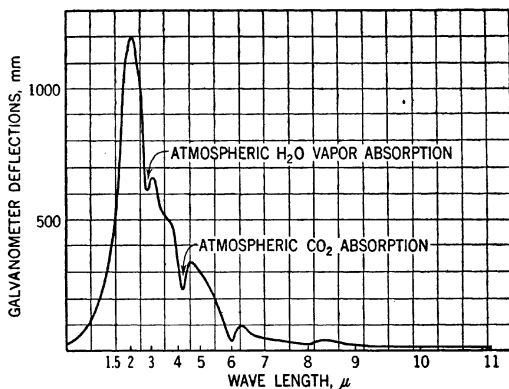


Fig. 36.—Emission of Nernst Glower as transmitted by a rock-salt prism spectrometer. (The wave-length scale is linear in prism settings.)

The spectra of these sources approximate black-body spectra in which the regular tenor is interrupted by the absorption of atmospheric gases, chiefly water vapor and carbon dioxide. In normal use, the maximum emission of the source is from about 1.2 to $2\ \mu$ according to the temperature, and there is a rapid fall in intensity toward the longer wave lengths (Fig. 36). The danger of the entry into the thermopile of spurious short-wave radiation at nominal longwave settings has already been mentioned; the use of filters and shutters in the attempt to eliminate impurity has been discussed (page 1344).

¹¹⁸ For example, A. Levaldi, *Rev. Sci. Instruments*, 11, 429 (1940).

¹¹⁹ L. G. Smith, *Rev. Sci. Instruments*, 13, 73 (1942).

The carbon dioxide and water vapor bands in the emission spectrum of the source cause difficulty in determining absorption accurately in installations whose optical path contains ordinary laboratory air. This is especially true at high resolution, when the structure of the atmospheric absorption bands causes extremely rapid variations of the intensity with wave length. A partial alleviation of this difficulty can be effected by means of placing desiccants and carbon dioxide absorbents in an air-tight spectrometer case, and by similar procedures in the rest of the optical path. Some workers have avoided the difficulty by evacuation of the spectrometer and as much as possible of the optical path.¹²⁰ The trouble is automatically eliminated in double-beam spectrometers (page 1364).

6. Cells

It is desirable to use a cell thick enough to cause about 40–80% absorption at the band maxima. For gases, a cell some six inches long is usually suitable; if the absorption of that thickness at atmospheric pressure is too great for accurate measurement at the maxima, the gas can be diluted with dry air, or partially pumped out (but see page 1368 for deviations from Beer's law in gases). Glass tubes about two inches in diameter with suitable inlet and outlet tubes, on which rock-salt windows are cemented by means of Glyptal or other cement, are often adequate. A gas cell usable at temperatures from 100° to 200° C. has been described by Smith.¹²¹

The general design for cells for organic liquids embodies an arrangement of flat, rock-salt plates separated by the required distance (say 0.05 to 0.5 mm.) by a spacer of mica or metal foil, held together by a framework by which the windows may be clamped to form a liquid-tight trough. Some arrangement for filling the cell conveniently is also required, for instance, a filling tube held tightly in a hole bored in one of the windows. A convenient cell for routine measurements has been described by Gildart and Wright¹²² who found that lead gaskets amalgamated with mercury allowed a tight joint to be made without the use of organic waxes. Filling the cells by means of a needle valve attached to one of the clamping plates with its outlet passing into a hole drilled into one of the rock-salt windows has been recommended.¹²³ A simple, easily constructed and simply manipulated cell is described by Colthup.^{123a}

¹²⁰ H. M. Randall and J. Strong, *Rev. Sci. Instruments*, **2**, 585 (1931).

¹²¹ L. G. Smith, *Rev. Sci. Instruments*, **13**, 65 (1942).

¹²² L. Gildart and N. Wright, *Rev. Sci. Instruments*, **12**, 204 (1941).

¹²³ N. D. Coggeshill, *Rev. Sci. Instruments*, **17**, 343 (1946).

^{123a} N. B. Colthup, *Rev. Sci. Instruments*, **18**, 64 (1947).

Accurate determinations of absorption coefficients necessitate an accurate knowledge of the cell thickness. Various methods of greater or less accuracy are available for this measurement, *e. g.*, measurement of the thickness of the spacer before assembly by means of a micrometer, or it may be possible to measure the thickness of the assembled cell by means of a vernier microscope. A cell can be calibrated by measuring the absorption of a substance of known extinction coefficient. The thickness of the air film in an absorption cell can be determined from observations of the interference pattern produced by the empty cell in the measuring beam.¹²⁴ Successive maxima and minima of intensity occur at wave lengths determined by the condition $2t = n\lambda/2$, or $n = 4t/\lambda = 4\nu t$, where n is a whole number, even for the maxima and odd for the minima. A plot of the successive maxima is therefore linear in the wave number, the slope being equal to four times the cell thickness. In a single-beam spectrometer, the interference pattern is observed against the rapidly varying profile of the energy curve of the source, and the maxima and minima cannot be determined with high accuracy, but in a double-beam instrument, great accuracy is possible.¹²⁵ For analytical purposes it is not usually necessary to know the cell thickness with a great accuracy; reproducibility of thickness of the cell when containing the sample and the standard, respectively, is usually the prime requirement.

7. Solvents

Usually the pure liquid or a mixture of liquids without extraneous solvent is used in infrared spectroscopy, but sometimes a solvent may be desired. Carbon disulfide is the most transparent common solvent in the infrared; it has a strong absorption band from about 6 to 7.8 μ , and others centered about 4.6, 11.7, and 13.4 μ ; elsewhere, out to 20 μ , its absorption is feeble. Carbon tetrachloride is also relatively transparent; it has strong bands at 12 to 14 μ , a fairly strong one at 6.5 μ , and feeble bands at 8 and 10 μ . There are no common polar solvents with extended regions of transparency from about 3 to 15 μ , although most substances have "windows" of restricted spectral extent in this region. Torkington and Thomson¹²⁶ give a list of solvents suitable for various regions, and further selections can be made from the compilations of spectra such as those of Coblenz,¹²⁷ and of Barnes, Liddel, and Williams.¹²⁸ Provided the layers are sufficiently thin,

¹²⁴ D. C. Smith and E. C. Miller, *J. Optical Soc. Am.*, **34**, 130 (1944).

¹²⁵ G. B. M. Sutherland and M. A. Willis, *Trans. Faraday Soc.*, **41**, 181 (1945).

¹²⁶ P. Torkington and W. H. Thomson, *Trans. Faraday Soc.*, **41**, 184 (1945).

¹²⁷ Coblenz, *Carnegie Inst. Wash. Pub.*, No. 35 (1905).

¹²⁸ R. B. Barnes, U. Liddel, and V. Z. Williams, *Ind. Eng. Chem., Anal. Ed.*, **15**, 659 (1943).

aqueous solutions can be examined in cells provided with silver chloride windows.^{128a}

8. Preliminary Adjustments

These consist of: setting the slits vertical (plumb bob) and at the focus of the collimating and "telescope" mirrors; placing the prism so that it is filled with the light reflected from the collimating mirror and, if the Wadsworth mounting is used, in the position of minimum deviation for some visible line, *e. g.*, the sodium or a mercury line; seeing that the Wadsworth condition (page 1340) is adhered to; and placing the "telescope" mirror so as to receive the refracted beam and to focus it on the exit slit.

To insure that the slits are at the focus of their respective concave mirrors, a simple method is to place an electric light bulb in front of them and view them thus illuminated by means of a telescope provided with cross hairs in the eyepiece. The cross hairs are first sharply focused by adjusting the eyepiece, and the telescope is set for parallel light by adjusting the distance between eyepiece and object glass until a distant object appears distinct when viewed through the eyepiece, and there is no parallax between the image and the cross hairs. The image of the illuminated slit formed by the concave mirror is then viewed by the telescope so set, and the distance between the slit and mirror adjusted until there is no parallax between the edge of the slit image and the cross hairs. The slit is then at the principal focus of the mirror.

9. Calibration

Wave-length calibration can be most simply effected by finding the settings for maximum absorption of known absorption bands. Convenient points for calibration of a rock-salt instrument are furnished by 20-cm. lengths of ammonia, carbon dioxide, and acetylene. Ammonia¹²⁹ has bands at 1.513, 1.967, 2.264, 2.988, 6.135, 10.322, and 10.710 μ ; the first strong line in the P branch, of ammonia at 11.008 μ , is sharp and suitable as a calibration point; carbon dioxide¹³⁰ has bands at 4.27 and 14.97 μ ; and acetylene¹³¹ at 4.406, 7.674, and 13.69 μ . The emission spectrum of the Bunsen burner flame has strong maxima at 2.8 μ (water vapor) and 4.4 μ (carbon dioxide) that can be used for calibration.

Standard wave lengths in the infrared have been determined by grating

^{128a} R. C. Gore, R. B. Barnes, and E. M. Petersen, "Symposium on Molecular Structure and Spectroscopy," Ohio State University, 1948.

¹²⁹ R. Robertson and J. T. Fox, *Proc. Roy. Soc. London*, **A120**, 161 (1928).

¹³⁰ E. F. Barker, *Astrophys. J.*, **55**, 391 (1922). P. E. Morton and E. F. Barker, *Phys. Rev.*, **41**, 293 (1932).

¹³¹ A. Levin and C. F. Meyer, *J. Optical Soc. Am.*, **16**, 137 (1928).

instruments, and for precise calibration of prism instruments it is important that the resolution of the prism and grating instrument be the same. Oetjen, Chao-Lan Kao, and Randall,⁸⁴ have measured the rotational lines in the absorption spectra of water vapor from 5 to 7.9 μ , of ammonia from 7.9 to 14 μ , and of carbon dioxide from 13.9 to 15.4 μ by means of a grating instrument working at the same resolution as a modern, rock-salt prism instrument. Many of these lines will normally appear in the source background when no provision is made to eliminate the effect of atmospheric absorption, and can thus serve as an internal standard, to check the constancy of calibration and make corrections if changes arise. Calibrations are unreliable over long periods. Unless special provision is made to maintain constancy of temperature or to compensate for temperature changes, the change of refractive index of the prism with temperature will be the main cause of shift in the calibration. For example, Oetjen, Chao-Lan Kao, and Randall found that a seasonal change of 9° C. in the temperature of the room produced a change as great as 0.14 μ in the wave length incident on the thermopile for a given prism setting. In addition, mechanical limitations of reproducibility and wear of moving parts produce changes in calibration, so that, for high accuracy, it is desirable to make a fiducial calibration in each run, or every day.

The procedure of mapping an infrared absorption spectrum will depend on the details of the equipment. If the operation is carried out manually, the most satisfactory method is to mount two similar cells, one containing the medium under examination, the other a blank, on a sliding carriage which allows them to be brought alternately into the beam at each spectrometer setting. The empty cells should first be tested against each other for equality of transmission throughout the spectral region of interest. The beam is arranged to pass axially through the cells without striking the walls; and the absorption coefficient of the medium can be calculated from the galvanometer deflections for the medium and blank after any corrections for reflection at the windows or for inequalities of transmission of the windows have been made. It is, of course, important to see that no change in the position of the image of the source on the entrance slit or on the thermopile occurs when one cell is substituted in the beam for the other.

In automatic recording of single-beam instruments, the simplest procedure is to scan the spectrum of the source alone, and then the spectrum with the absorbing sample in position, and to obtain, at least at the beginning and end of the run, an indication of the zero when no energy is received by the thermopile. Over an extended wave-length region, several adjustments of slit width will be required in order to maintain the energy at a measurable value. Inconstancy in the emission of the source and in drift of the receiver introduce inaccuracies. With thermopiles of the fine-

wire type, a period of about 30 to 50 minutes is required to obtain the two energy curves and the zero points from 3 to 18 or 25 μ , although with fast thermopiles and bolometers this time can be considerably shortened.

It is also possible to arrange an automatic system so that the measurements are made at discrete wave lengths, for each of which a zero representing no reception of energy is obtained.⁸⁷ Effects of thermopile drift and, to a large extent, of inconstancy in the source are thus eliminated, but at the expense of an increase in the time required to cover the spectrum.

A serious drawback with respect to speed of obtaining results is the time required to convert the two records of the energy-wave-length distribution for the source and for the sample into values of the percentage transmission. This must be done point by point along the wave-length axis, and is more time-consuming than the recording of the energy curves themselves. Various devices have been suggested to facilitate the conversion of the energy curves to transmittance values. Special scales can be used to permit reading off the transmittance from the recorded energies at any wave length on the source and sample curves,¹³² and Fuoss and Mead¹³³ describe an ingenious arrangement by which the various points on the sample curve representing the intensity of the light transmitted by the samples are made to select a potential difference, E , proportional to the corresponding intensity. This potential is fed into a resistance, R , proportional to the intensity of the source at the same wave length, and the resulting current, $i = E/R$, is then proportional to the transmittance of the sample. This current is fed into the recorder attached to the spectrometer, which therefore traces the transmittance-wave-length curve as the source and sample curves are traversed by pointers which select the conjugate resistance and potential differences. If the reading for the energy in the source is maintained at a fixed value, 100, at all wave lengths, then the reading for the sample will automatically be the percentage transmission. White¹³⁴ has described the use of a variable attenuator connected between the amplifier and recorder of a Perkin-Elmer spectrometer so that the overall amplification of the signal fed into the recorder can be changed automatically and continuously at a predetermined rate as the spectrum is scanned. The source curve can therefore be kept nearly flat and the sample curve will automatically give the transmittance values.

In order to obtain enough energy to cause the recording instrument to furnish a measurable response, it is often necessary in infrared spectroscopy to use slit widths that greatly reduce the resolving power below the theoretical. The considerations of slit widths recounted on page 1303 are

¹³² H. A. Willis and A. R. Philpotts, *Trans. Faraday Soc.*, **41**, 187 (1945).

¹³³ R. M. Fuoss and D. M. Mead, *Rev. Sci. Instruments*, **16**, 227 (1945).

¹³⁴ J. U. White, *J. Optical Soc. Am.*, **36**, 362 (1946).

particularly important. In reporting infrared absorption spectra, the slit width, measured in terms of the spectral interval in wave length or frequency units included within the slit, should be included.

For a prism instrument at minimum deviation in a Littrow mount the frequency interval, $\Delta\nu$, between two monochromatic lines of equal intensity which the instrument just fails to resolve is given by:¹³⁵

$$\Delta\nu(\text{cm.}^{-1}) = \frac{\nu^2(1 - n^2 \sin^2 \alpha/2)^{1/2}}{4 \sin \alpha/2 \cdot dn/d\lambda} \cdot \frac{S_1 + S_2}{f} + \frac{\nu}{2b \, dn/d\lambda} \quad (24)$$

where ν is the frequency in cm.^{-1} , n the refractive index of the prism for light of frequency ν , α the prism angle, $dn/d\lambda$ the dispersion of the prism in cm.^{-1} , S_1 and S_2 the two slit widths in cm. , f the focal length of the spectrometer mirror in cm. , and b the effective base of the prism in cm. The second term in this expression is the limiting resolving power according to Rayleigh's criterion (page 1261) while the first is the additional impurity introduced by finite slit widths.

TABLE IX
RELATION BETWEEN GEOMETRIC AND SPECTROSCOPIC SLIT-WIDTHS FOR PERKIN-ELMER SPECTROMETER¹³⁵

ν , cm.^{-1}	λ , μ	Slit width, mm.	Prism	Slit width, frequency, cm.^{-1}		
				A	B	Total
3132	3.2	0.04	LiF	3.5	1.4	4.9
1618	6.3	0.055	NaCl	4.4	2.6	7.0
950	10.5	0.120	NaCl	1.9	0.9	2.8
500	20	0.67	KBr	3.5	0.6	4.1

In table IX are summarized, as examples of the relation between the geometric slit width and the slit width in frequency units, data given by Barnes *et al.*,¹³⁵ for a Perkin-Elmer spectrometer. In this table, the column headed *A* gives the slit widths in frequency units as computed according to the first term in the expression for constant-deviation slit widths, and that headed *B* gives the limiting slit width according to Rayleigh's criterion. Since the Perkin-Elmer spectrometer is not used at minimum deviation at all wave lengths, the actual values are slightly different from those tabulated.

10. Double-Beam Infrared Spectrometers

A recent development in infrared spectroscopy, and one of the utmost importance for the future, is the attack on the problem of the double-beam

¹³⁵ R. B. Barnes, R. S. McDonald, V. Z. Williams, and R. F. Kincaid, *J. Applied Phys.*, **16**, 77 (1945).

spectrometer. The principle of this instrument is essentially the same as that of the double-beam spectrophotometers usually used in visible and u.v. spectroscopy (p. 1300). Light from a source is accurately divided into two equivalent beams, one of which passes through the sample, the other through a reference substance, say air or an equivalent thickness of solvent. The intensity of the beam passing through the standard can be equalized by a variable aperture device to that of the beam passing through the sample and the position of some kind of pointer indicating the effective aperture in the standard beam at photometric balance is a measure of the percentage of transmission of the sample. This is the normal procedure in the visible and ultraviolet regions of the spectrum. Alternatively, a receiver which indicates the ratio of the intensities in the two beams may be used. When the operation of such an instrument is speedy, reliable, and automatic we are within sight of the ideal infrared spectrophotometer.

In the infrared, circumstances are such that the double-beam technique confers, relatively to the single beam, even greater advantages than in the visible or ultraviolet. In addition to eliminating the effects of inconstancy of the source, some characteristic infrared bugbears are also eliminated, such as the effects of atmospheric absorption or of absorption bands in the optics of the instrument, and thermopile or bolometer drift; also, since the final record is one of percentage of transmission throughout the spectrum, the need for the laborious conversion of the separate energy readings for source and sample in single-beam technique automatically disappears.

The principles of double-beam infrared spectrophotometry can be illustrated from the method used by Hardy and Ryer¹³⁶ in the first published account of this type of instrument. Two equal beams of light are selected from the radiation of a Nernst filament and are focused one above the other at the entrance slit of the spectrometer, which can be of the conventional type. In one beam is placed the sample, in the other a variable aperture. The two beams pass through the spectrometer, and are finally brought to focus on two identical thermopiles one above the other, one of which therefore responds to the radiation which has passed through the sample, the other to that which has passed through the reference substance. The two thermocouples are connected in opposition, so that, when the sample is withdrawn and only the reference substance (for instance, air) is present in the beam, no deflection is indicated at any wave length. If the sample now absorbs more than the reference substance at some wave length, a deflection will be indicated. Equality of intensity in the two beams can now be restored by partially closing the variable aperture in the standard beam; the position of the aperture is recorded against the position of the prism, so

¹³⁶ J. D. Hardy and A. I. Ryer, *Phys. Rev.*, **55**, 1112 (1939).

that, as the spectrum is traversed, the transmission-wave-length curve is obtained.

The instrument of Hardy and Ryer was not fully automatic, the balance being performed manually. An automatic instrument utilizing the principle of balanced beams has been described by Wright and Herscher.¹³⁷ A Littrow spectrometer is employed, similar to that of figure 30. The receiver is a single nickel strip bolometer capable of responding to radiation modulated at 6 cycles per second. The output of the bolometer is connected through a transformer to a 4-stage tuned feedback amplifier, for details of which the original article must be consulted. The mode of action of the photometer is like that of the *General Electric* automatic spectrophotometer for the visible (p. 1323). Two beams, 1 and 2 (Fig. 37),

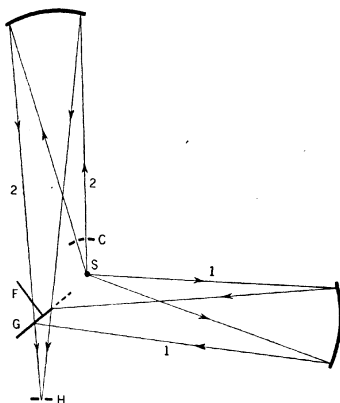


Fig. 37.—External arrangement for double-beam spectrometer of Wright and Herscher.¹³⁷

from a Globar, *S*, are made to fall alternately on entrance slit *H* of the spectrometer. The sample is placed in 1, and the standard in 2. *G* is a semicircular-sector mirror which rotates on shaft *F* at 6 cycles per second. When the mirror is above the shaft, the sample beam 1 is reflected into the entrance slit of the spectrometer, and 2 is blocked off: when the mirror is below the shaft, the standard beam 2 enters the slit and 1 passes to the left and is lost. In the standard beam, near the source, is placed a variable diaphragm, *C*. This has the form of a comb with 4 or 6 triangular teeth which can be advanced across the beam by means of a worm-gear arrangement connected to the balancing motor of a pen recorder, transmitting

¹³⁷ N. Wright and L. W. Herscher, *J. Optical Soc. Am.*, **37**, 211 (1947).

more or less of the beam according to its position. The comb, and not the Globar, is imaged at the slit, and the spectrometer aperture is filled at all positions of the comb.

If the beams are unequal, the bolometer will be subjected to a "flicker" at 6 cycles per second, and its output will alternate at this frequency. The phase will depend on which of the beams is the more intense. This 6-cycle alternating electromotive force is amplified by the tuned amplifier; the output of the amplifier is, in turn, rectified by a cam switch mounted on the shaft of the sector motor. The resulting d.-c. voltage, whose polarity is determined by whichever of the two beams is the more intense, passes to the recorder, a recording milliammeter of the *Brown Instrument Co.*, where after further amplification it actuates the balancing motor of the recorder. This is connected to the variable diaphragm by the worm gear already referred to, so that, as it operates the recording pen, it moves the diaphragm simultaneously in such a direction as to balance the two radiation beams. It will be observed that linearity of the bolometer response is not assumed in this method, and that variation in the amplifier gain has no effect. The spectrum is scanned in the usual way by motor-driven rotation of the Littrow mirror. The motion of the recorder paper is synchronized with that of the prism by means of a pair of Selsyn motors. The wave-length drive is also coupled to a linear-gear mechanism which continuously varies the slit widths so as to maintain the energy in the standard beam approximately constant throughout the spectrum. Thirteen minutes are required to record from 2 to 15 μ . *Baird Associates*, Cambridge, Mass., manufacture an infrared spectrophotometer embodying the double-beam principle.

The alternative method of recording the ratio of the sample to the standard beam is described by Wild.¹³⁸ As on the instrument of Hardy and Ryer,¹³⁶ the standard and sample beams follow slightly different paths through the spectrometer and fall on separate matched thermopiles. The output of each thermocouple is amplified by an amplifier of the "breaker" type and the amplified voltages are compared by a recording potentiometer. Adjustment of slit width and all other readjustments during a run are automatic.

The spectrophotometry of microscopic samples can be carried out by combining a Burch reflection microscope with a spectrophotometer.^{138a} By projecting a magnified image from the microscope on the slit of a Perkin-Elmer spectrometer, Barer, Cole, and Thompson have obtained excellent infrared spectra of samples of biologically important materials in amounts

¹³⁸ R. F. Wild, *Rev. Sci. Instruments*, **18**, 436 (1947).

^{138a} R. Barer, A. R. H. Cole, and H. W. Thompson, *Nature*, **163**, 198 (1949).

of 10^{-7} to 10^{-8} g. This would seem an important advance in the spectroscopy of difficultly obtainable material.^{138b}

11. Beer's Law in the Infrared^{139, 140}

It has long been known that the measured absorption of certain *gases* for wave lengths within their infrared absorption bands does not depend merely on the number of absorbing molecules in the optical path, but also on the total pressure, even if part of the total pressure is contributed by

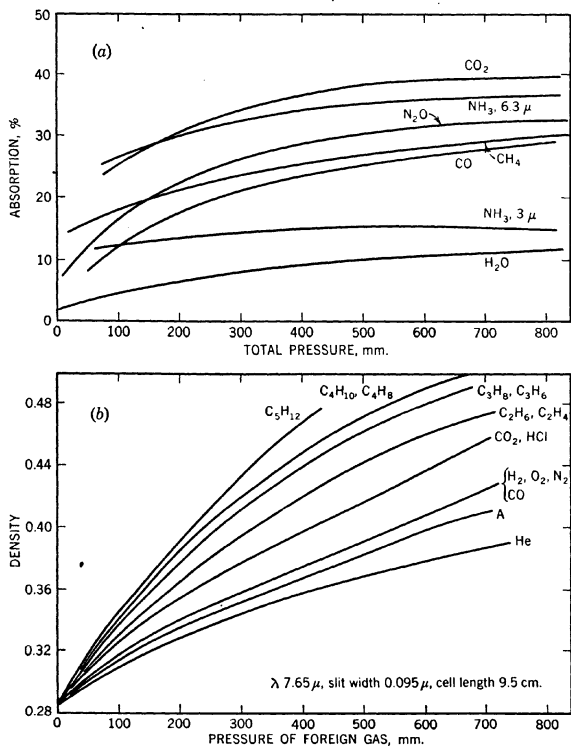


Fig. 38.—Pressure effect in gases: (a) effect of air, H_2 , and O_2 on various absorbing gases; (b) effect of different foreign gases on CH_4 .

^{138b} See also S. H. Wollman, *Rev. Sci. Instruments*, **20**, 220 (1949).

¹³⁹ J. R. Nielsen, V. Thornton, and E. B. Dale, *Revs. Modern Phys.*, **16**, 307 (1944).

¹⁴⁰ N. D. Coggeshill and E. L. Saier, *J. Chem. Phys.*, **15**, 65 (1947).

chemically inert transparent gases. Gases whose absorptions at constant partial pressure are increased by adding foreign gases are also found to deviate from Lambert's law (p. 1295), the optical density at constant pressure of the absorbing gas increasing less rapidly than linearly with the column length.

The magnitude of the deviations from Beer's law is shown in figure 38a. Each curve pertains to a fixed value of the product of partial pressure and the cell length for the corresponding infrared-absorbing gas (not necessarily the same product for different gases). The absorption is shown to increase with increasing pressure of the transparent foreign gases hydrogen, oxygen, and air. Except for minor deviations due to variation from the ideal gas laws, the number of molecules of any one absorbing gas is constant and, if Beer's law were followed, the curves would be straight lines parallel to the axis of total pressure. Instead, as the pressure of foreign gas is increased, the absorption of the fixed amount of infrared-absorbing gas increases, at first rapidly, then, at a sufficiently high total pressure, tends toward a constant value. If the critical total pressure is maintained, the density at constant cell thickness, if measured for sufficiently monochromatic light, increases linearly with the partial pressure of the absorbing gas.

Different foreign gases have different effects on a given absorbing gas as is illustrated in figure 38b for methane, from the work of Coggeshill and Saier.¹⁴⁰ Several of the foreign gases in this example have infrared absorption bands, but their absorption at the wave length considered is small, and corrections were made on the assumption that their contribution to the total absorption was given by Beer's law. Any error caused by this assumption would seem to be of second order. The gases which show strong dependence of transmission on the presence of foreign gases have low moments of inertia. Vapors such as methyl alcohol, ethyl ether, and benzene show little sensitivity to this effect. A small moment of inertia corresponds to relatively open spacing of the rotational lines of which the infrared absorption bands of gases are composed; the pressure effect of foreign gases is therefore most marked when the absorbing gas has appreciable spacing (as indicated by instruments of high resolving power) between the rotational lines in its infrared bands.

It has been shown that in the infrared the main cause of the finite width of the individual rotational lines in a gas is the perturbation or phase change in the molecular vibrations that accompanies collision with another molecule—the *collision damping* first investigated theoretically by Lorentz. The distribution of absorption intensity with frequency, $A(\nu)$, over an individual line whose breadth is determined solely by collision damping has the form:

$$A(\nu) = \frac{Knlf}{4\pi^2(\nu - \nu_0)^2 + f^2} \quad (25)$$

where K is a constant for the individual line, n the number of absorbing molecules per unit volume, l the length of the absorbing column, ν_0 the frequency at the maximum of the line, and f the mean frequency of collisions experienced by a single absorbing molecule. Addition of a foreign nonabsorbing gas increases f in a manner depending on the temperature, masses, and the nature of the collision, and the equation shows that the proportion of the absorption at frequencies removed from the maximum increases, *i. e.*, the line broadens.

According to kinetic theory:

$$f = (2kT/\pi)^{1/2} n \sigma^2 [(m + M)/mM]^{1/2}$$

where T is the absolute temperature, k the Boltzmann constant, n the number of molecules per cc., m the mass of the absorbing gas molecule, M the mass of the foreign molecules, and σ the "optical collision diameter," which turns out usually to be several times greater than the normal "gas kinetic diameter" deduced from viscosity, etc. From studies on the pressure effects of gases on infrared bands, information can be obtained on the optical collision diameters and the intermolecular forces.

In addition to true effects of increased absorption over that determined by Beer's law produced by pressure broadening, apparent deviations may arise from the use of a slit such that great variation in the value of the extinction coefficient occurs over the frequency interval covered by the slit (page 1303). This circumstance will arise when a very narrow band is examined by a slit width equal to an appreciable portion of the band width, for example, the Q branches (page 1335) of molecules with large moments of inertia. In this case, the measured density at constant cell length increases less rapidly than linearly with the concentration of the gas.

Detailed analysis of the pressure effects in gases requires consideration of both the pressure-broadening and the slit-width factors. Theoretical curves have been obtained by Nielsen *et al.*¹³⁹ which reproduce the observed features, for example, an increase in density at constant product of partial pressure of absorbing gas and cell length with total pressure up to a limiting total pressure, at which the density of the absorbing gas increases linearly with its partial pressure. These facts have important practical interest in any infrared study involving gases. For example, if relative intensities of bands of a gas at different wave lengths are under investigation, it is not permissible to dilute the absorbing gas with different amounts of air for the sake of obtaining a suitable transmission for measurement. These effects are especially important in the quantitative analysis of gas mixtures.

As was discussed on page 1335, a common type of infrared band is composed of unresolved *P*, *Q*, and *R* branches, of which the *Q* branch is very narrow, and the others broader. The sharp *Q* branches are often found to deviate from Beer's law, while the lateral branches may conform to the law and show absorption relatively independent of slit width.¹³⁹ These *Q* branches are often the most intense bands and offer valuable characteristics for qualitative analysis, but because of apparent deviations from Beer's law may not be the most suitable bands for quantitative analysis.

In *liquids* and *solutions* rotational structure almost invariably disappears; in solution the critical concentration of foreign molecules at which the density increases linearly with the concentration of the absorbing molecules always exists. Other factors, of course, causing true deviations from Beer's law may come into play, such as changes of the molecular species as a result of dimerization or polymerization, or strong interaction between solvent and solute. Again, apparent deviations may originate in the use of slit widths that cover an appreciable portion of the absorption band.

12. Grating Spectrometers

Most of the detailed analysis of infrared spectra has been done with grating spectrometers of the echelette type. Such instruments must be used in conjunction with prism monochromators or with filters to effect a preliminary isolation from higher orders of the wave-length region to be studied. Several gratings are required to cover the region from 3 to 15 μ ; and it seems likely that, for most purposes of interest to the organic chemist, a good prism instrument would be adequate and considerably less troublesome to use, particularly with automatic recording.

Some details of the procedure for quantitative analysis by means of infrared spectra and other applications to organic chemistry are given in Section IX (page 1384).

VII. RAMAN SPECTROSCOPY

1. General

Allied to infrared absorption spectra in their connection with molecular vibrations, but differing rather profoundly in their mode of excitation, are the Raman spectra of substances. They are nearly always excited by visible or ultraviolet light, but no absorption in the ordinary sense is involved. In general, a transparent medium illuminated with monochromatic light scatters both light at the same wave length as that of the incident light, and also light of modified wave lengths, the frequency difference between the incident and modified light being related to vibrational and rotational frequencies in the molecule. The modified scattering gives rise

to the Raman spectra. For example, the spectrum of the light scattered by liquid carbon tetrachloride illuminated by a line source contains, in addition to the exciting lines, three relatively strong lines displaced 218, 314, and 459 cm^{-1} to lower frequencies, and a feeble pair displaced 762 and 790 cm^{-1} (Fig. 39). These frequency differences between incident and scattered light are customarily called Raman frequencies and are independent of the frequency of the exciting line. The set of Raman frequencies of a substance constitutes its Raman spectrum, characteristic of the substance under given conditions. There may be present, in the scattered spectrum, lines displaced to higher frequencies than the exciting lines, by the same amount as those to lower frequencies, the so-called anti-Stokes lines. The intensity of a given Raman frequency increases inversely as a high power of the exciting wave length. Blue and violet exciting lines are therefore

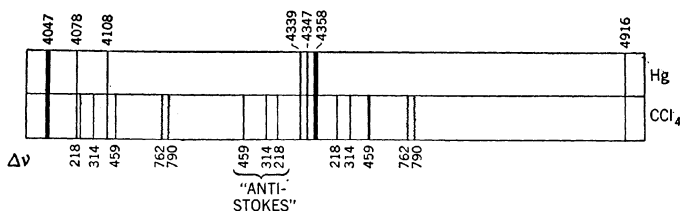


Fig. 39.—Raman spectrum of carbon tetrachloride excited by Hg 4358 and 4047 Å.

most effective in the visible; and, when photochemical changes, fluorescence, or absorption of the compound do not prevent their use, ultraviolet lines such as the 2537 Å. line from a mercury resonance tube are particularly effective.

The theory of the Raman effect shows that the Raman frequencies are closely related to frequencies of vibration within the molecule. In its Raman interaction with the molecule, part of the energy of the exciting photon is in effect utilized by the molecule to excite a vibration, and the remainder escapes as a photon of energy lower by the amount taken up by the molecule. The frequency difference between the exciting and scattered radiation multiplied by Planck's constant, $h\Delta\nu$, is therefore equal to the quantum of vibration, $h\bar{\nu}$, where $\bar{\nu}$ is the mechanical frequency of the vibration considered as a simple harmonic oscillator. It follows that $\Delta\bar{\nu} = \bar{\nu}$, or, in wave numbers instead of in frequency units, $\Delta\nu = \nu$. In a similar manner, the anti-Stokes lines are produced by the addition of the energy of a vibrational quantum of the molecule to the incident photon, which escapes with a higher frequency. The Raman spectrum is, therefore, to some degree a manifestation in visible or ultraviolet light of the molecular processes which cause absorption in the infrared, but it is to be noted that

not all infrared frequencies have their Raman counterpart and vice versa. Reference must be made to specialized works, such as that of Hibben,¹⁴¹ for the selection rules governing the relations between molecular vibrations and the Raman and infrared frequencies; but one important and easily remembered rule may be mentioned—that, in molecules with a center of symmetry, molecular vibrations in which motion of the nuclei is symmetrical to the center of symmetry are excited in Raman scattering and not by infrared absorption, and, conversely, vibrations antisymmetrical to the center are excited by infrared absorption but not in Raman scattering.

Raman lines excited by unpolarized light may be more or less polarized, and Raman lines excited by polarized light may be depolarized. In general, the strong lines associated with symmetrical molecular vibrations are the most highly polarized. Reference must again be made to the treatises (see page 1397) on the Raman effect for the relation between the degree of depolarization and molecular structure.

The determination of a Raman spectrum therefore consists in the evaluation of the wave lengths or frequencies of the lines, their intensities, and state of polarization. In investigations of molecular structure, all three factors must be known; but there are many applications to analysis, identification, etc., in which the measurement of polarization can be dispensed with.

2. Light Sources and Filters

General requirements are a high-intensity line spectral source, preferably with a few sharp lines in the blue, violet, or ultraviolet, free from continuous emission, a high-aperture spectrograph of reasonable dispersion, and avoidance of all factors, like fluorescence of the material under investigation, reflection, and scattering of the source by the tube holding the material, which may bury the Raman lines in a continuous background. Since many organic substances absorb ultraviolet light, visible light will usually be most convenient for excitation, and the mercury arcs are by far the most common source. Quartz arcs can be run at much higher intensity than glass arcs. Batteries of mercury arcs of the H type (page 1283) are also often used (Fig. 41). Besides ultraviolet lines, the mercury arc emits strong lines at 4047 Å. in the violet, 4358 Å. in the blue, accompanied by weak lines at 4348 and 4339 Å., a rather weak line at 4916 Å. and strong lines at 5461 Å. in the green and 5769 and 5790 Å. in the yellow. The possibility of scattering by the short-wave companions of 4358 should not be overlooked when that line is used for excitation. Between wave lengths 4358 and 4916 Å. is a region free from lines, in which fall most of the Raman lines scattered by 4358. High-pressure mercury arcs, however, emit a few

¹⁴¹ J. H. Hibben, *The Raman Effect and Its Applications*. Reinhold, New York, 1939.

diffuse bands in this region, whose interpretation as scattered radiation should be guarded against. The continuous spectrum emitted by high-pressure mercury arcs on the long wave side of 4358 line can also be troublesome as tending to suppress feeble Raman lines, but can be removed by filters (see next paragraph). The strong green line at 5461 Å. is a possible source if the substance absorbs in the blue, but the rapidly decreasing intensity of Raman scattering with increasing wave length of the exciting vibration makes the use of this line much more difficult than that of the blue or violet lines.

If unfiltered light of a medium pressure Hg arc is used to excite scattering and the spectra are recorded on photographic plates without dye sensitization, the Raman lines observed are those excited by the violet and blue Hg lines. Usually it is not difficult to assign the Raman lines to their respective excitors, but in cases of doubt, one of the Hg lines should be removed by a filter and monochromatic excitation secured. A solution of iodine in carbon tetrachloride is a convenient filter for wave length 4047 Å. Wood¹⁴² recommends the preparation of a saturated solution, which at 1:10 dilution will reduce 4358 Å. so as to make it ineffective. A more dilute solution, 1:35, transmits both 4047 and 4358 Å., but suppresses the continuous background of the high-pressure arc on the long-wave side. Another filter useful for isolation of 4358 Å., practically free from 4047 and continuous background, is a 3-cm. thickness of 2% alcoholic *p*-nitrotoluene and 1 part in 50,000 of rhodamine 5 GDN Extra.¹⁴³ A concentrated solution of sodium nitrite absorbs the ultraviolet and 4047 Å., transmitting the rest of the mercury spectrum. Removal of ultraviolet light to prevent fluorescence can be effected by the glass Corning filter Noviol O (*Corning Glass Works*, Corning), by the Wratten filter 2A (*Eastman Kodak Co.*, Rochester), by a solution of sodium nitrite, by alcoholic *m*-dinitrobenzene or *p*-nitrotoluene. The Wratten filter can be conveniently wrapped around the Raman tube.

The green line of mercury at 5461 can be isolated by a solution of 0.15 g. malachite green and 0.3 g. tartrazine in 1 l. of water. As already mentioned, the short-wave length mercury line 2537 Å. makes it particularly valuable as a Raman excitor when absorption or fluorescence does not prohibit its use. In the relatively few cases in which this mode of excitation can be used for organic compounds (paraffins, simple olefins), the commercial rare-gas high-voltage resonance lamps form excellent sources.¹⁴⁴ These are obtainable in spirals which can be wrapped round the Raman tube.

¹⁴² R. W. Wood, *Physical Optics*. 3rd ed., Macmillan, New York, 1934, p. 449.

¹⁴³ J. T. Edsall and E. B. Wilson, *J. Chem. Phys.*, **6**, 125 (1938).

¹⁴⁴ Supplied, for example, by the *Ultra-violet Technical Laboratories*, Los Angeles, Calif., or *The Hanovia Chemical & Mfg. Co.*, Newark, N. J.

3. Arrangement of Source and Raman Tube

The principle is to illuminate the medium under investigation from the side by as intense a source as possible and to photograph the spectrum of the radiation scattered at right angles to the direction of incidence of the exciting light, avoiding the entry into the slit of the spectrograph of all radiation except that which comes from the scattering volume.

The freshly distilled liquid is contained in a tube, conveniently some 4 or 5 in. long and $\frac{1}{2}$ to $\frac{3}{4}$ in. in diameter, which is often drawn out to a blackened horn at the rear in order to avoid the reflection of light from the glass end into the spectrograph. Completely blackening the rear end of the tube and diaphragming, by black paint or otherwise, the front

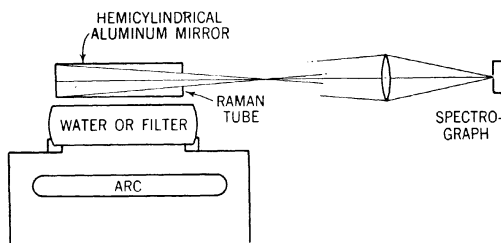


Fig. 40.—Arrangement for Raman spectrum.

end, so that the walls of the tube are not seen by an eye placed at the edge of the condensing lens focusing the scattered light on the slit of the spectrograph, secures the absence in the scattered beam entering the spectrograph of most of the light reflected by the walls.

The detailed arrangement of source and scattering tube naturally depends on the nature of the material under examination, but a simple experimental setup is shown in figure 40. The arc is contained in a metal box with a slit for the passage of the light, at which is mounted a glass tube, 1.5 to 2 inches in diameter, containing water or a filtering medium, acting as a cylindrical lens to project an image of the arc at the center of the Raman tube. Other flat filters can also be introduced. Round the far side of the Raman tube is wrapped a piece of polished aluminum to reflect the exciting light again into the medium. The front end of the Raman tube is diaphragmed as described before, and a reduced image of the tube focused on the slit of the spectrograph by a lens of about 20-cm. focal length placed about 1 m. or so from the tube. It is helpful in suppressing background to enclose the whole path from Raman tube to slit in easily mountable and dismountable cardboard tubes.

Figure 41 contains a diagrammatic sketch of a "light furnace" suitable for illumination at high intensity.¹⁴⁵ The Raman tube, *A*, is placed along the axis of a cylindrical, chromium-plated reflector, *F*, connected by a cone-shaped adapter to a blower for the removal of hot air. Eight mercury lamps, *E*, General Electric type A-H2 (250 watts), arranged in a circle, provide the illumination. The authors report that the type A-H11, similar to the A-H2, except for a reduction in the amount of mercury used, gives

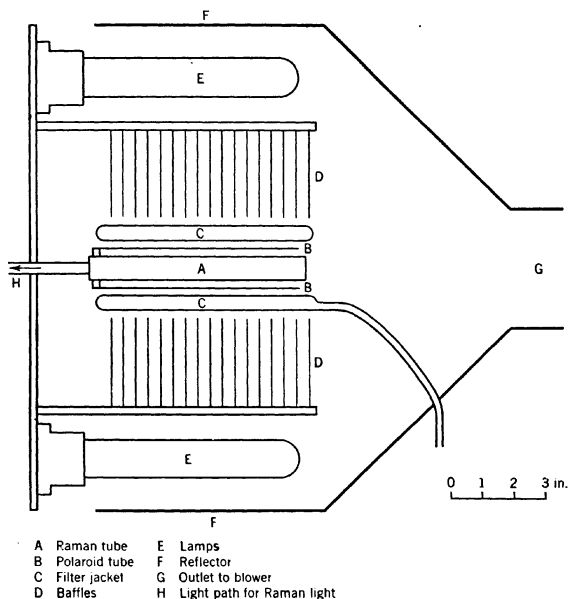


Fig. 41. "Light furnace" for polarization experiments (after Crawford and Horwitz).¹⁴⁵

relatively less continuous background than the A-H2, but at the expense of a loss of half the operating intensity. The baffles, *D*, and the Polaroid filter, *B*, are used in connection with polarization experiments; if only the Raman frequencies are of interest, they can be eliminated, and the dimensions changed so as to allow closer approach of the lamps to the Raman cell with some gain in intensity. An alcoholic solution of Rhodamine 5 GDN extra is circulated through the cylindrical filter jacket, *C*, and a cooling coil immersed in running tap water, for the isolation of the line at 4358

¹⁴⁵ B. L. Crawford and W. Horwitz, *J. Chem. Phys.*, **15**, 268 (1947).

Å. and the suppression of continuous background; ultraviolet light is excluded by a Wratten 2A gelatin filter in the position *B*.

This arrangement, including the baffles and Polaroid filter, was capable of producing a photographic density of 1.0 in the Raman line of carbon tetrachloride at 314 cm.^{-1} , in 30 minutes' exposure; 13 minutes brought the benzene line at 992 cm.^{-1} to this density. A 3-prism Steinheil spectrograph, $f/3$, with a dispersion of 32 Å. per mm. at 4358 Å. was used, with a slit width of 0.1 mm.

A very intense illuminating arrangement is described by Kirby-Smith and Bonner.¹⁴⁶ It is relatively easy to obtain good Raman spectra from tubes about 3 cm. long and 1 cm. in diameter, and even from smaller tubes. Micromethods, employing down to 0.1 ml. , have been described by Grassmann¹⁴⁷ and by Dadieu.¹⁴⁸ Occasionally Raman spectra free from continuous background can be obtained only by continuously renewing the liquid in the scattering tube by means of a circulating distillation system. The Raman tube is made the receiver in a distillation setup and provided with a return tube carrying the condensate back to the distilling flask.

Raman spectra have been obtained in all states of aggregation, but the investigation of liquids or concentrated liquid solutions is by far the easiest. For many of the problems of organic chemistry, the spectra of liquids are satisfactory, and reference must be made to the special treatises (page 1397) for the details of the methods with gases and solids.

4. Alignment of Raman Tube and Spectrograph

The alignment of Raman tube and condensing lens with the slit and collimator axis should be carefully performed. The distance between the front window of the Raman tube and the slit will be about 125 cm. or more. According to Wood's method,¹⁴² the position for the slit is first marked by looking from the proper distance along the axis of the illuminated Raman tube, and bringing a pin held in a stand before the eye. The slit is then brought up to the pinhead; and, on looking into the spectrograph from the position of the plate, one should see the end of the tube as a small circle of light in the camera lens. The spectrograph is moved, keeping the position of the slit fixed at the pinhead, until the circle of light is in the middle of the camera lens. The condensing lens is then brought into position by placing it so as to focus the image of the Raman tube face on the slit. There are usually features about the end of the tube which reflect enough light from the source of Raman excitation to form a recognizable image at

¹⁴⁶ J. S. Kirby-Smith and L. G. Bonner, *J. Chem. Phys.*, **7**, 880 (1939).

¹⁴⁷ P. Grassmann, *Z. Physik*, **72**, 240 (1936).

¹⁴⁸ A. Dadieu, *Z. angew. Chem.*, **49**, 344 (1936).

the slit; a piece of white paper half covering the slit is helpful in making the image visible. If necessary, the front of the Raman tube can be covered with a piece of white paper and illuminated with a lamp to obtain an image that can be focused at the slit. Bright parts of the image of the Raman tube due to reflection of the source must not be allowed to enter the slit; they can either be diaphragmed off at the tube, after alignment, or by means of a wedge diaphragm at the slit.

5. Spectrographs for Raman Effect

The requirements here are speed and, usually, moderate dispersion. The glass constant-deviation instruments previously mentioned (page 1254) are serviceable, though naturally not as fast as the high-aperture high-resolution instruments introduced by the principal manufacturers since the discovery of the effect. A discussion of spectrographs for the Raman effect is in a paper by Nielsen.^{149*}

As in other branches of spectroscopy, it seems likely that in studies of the Raman effect there will be growing displacement of photographic by photoelectric methods, especially for the measurement of intensities. Under proper conditions, the currents yielded by vacuum photocells and by electron multiplier tubes are directly proportional to the incident intensity, and the troublesome intensity calibration of the photographic plate is eliminated. The only calibration necessary is the wave-length sensitivity.¹⁵⁰⁻¹⁵³ The low intensity of Raman radiation necessitates very high amplification of the photocurrents from a photoelectric cell, and it is better practice to use multiplier tubes and an amplifier of moderate gain which can be constructed from standard parts (page 1320). The use of multiplier tubes in Raman spectroscopy was introduced by Rank, Pfister, and Coleman,¹⁵⁰ and has been applied to the determination of the relative intensities of Raman lines in a given substance, to the relative intensity of Stokes and anti-Stokes lines, and to depolarization measurements. The circuit requirements are a voltage supply for the multiplier constant to 0.1%, and a stable linear amplifier. The tube is moved slowly across the focal plane of the spectrograph, and a photographic record is made of the deflections of a galvanometer in the output circuit of the amplifier as the spectrum is

* A complete set of equipment for Raman spectroscopy, including a high-aperture spectrograph, and a high-intensity source, is manufactured by *Lane-Wells Company*, Pasadena, Calif.

¹⁴⁹ J. R. Nielsen, *J. Optical Soc. Am.*, **20**, 701 (1930).

¹⁵⁰ D. H. Rank, R. J. Pfister, and P. O. Coleman, *J. Optical Soc. Am.*, **32**, 390 (1942).

¹⁵¹ D. H. Rank, R. J. Pfister, and H. H. Grimm, *ibid.*, **33**, 31 (1943).

¹⁵² D. H. Rank and R. V. Wiegand, *ibid.*, **36**, 325 (1946).

¹⁵³ Jen-Yuan Chien and P. Bender, *J. Chem. Phys.*, **15**, 376 (1947).

scanned. Figure 42 illustrates records obtained by Jen-Yuan Chien and Bender¹⁵³ using a Steinheil spectrograph with 3 glass prisms, and of focal length 19.5 cm. and aperture ratio $f/3$. The deflections for the peaks of the Raman lines can be converted to relative intensities when the spectral sensitivity of the multiplier tube is known.

In using the Raman effect as a means of quantitative chemical analysis, the variation of intensity of the Raman lines with concentration is utilized. To eliminate the effect of varying intensity of the source during exposure, it is best to employ an "internal standard," for example, by comparing

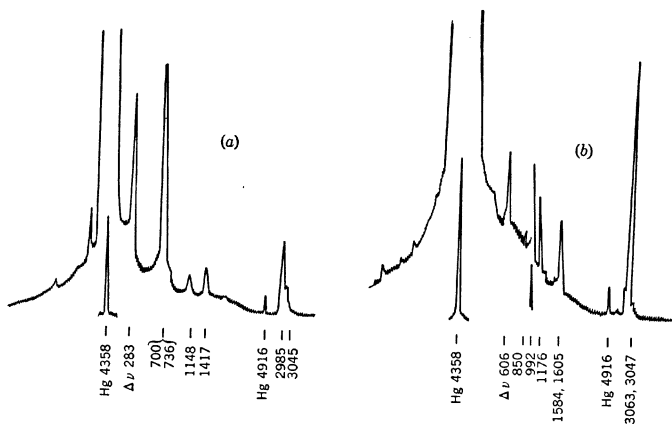


Fig. 42.—Raman spectra recorded by multiplier tube (Jen-Yuan Chien and Bender¹⁵³): (a) dichloromethane; (b) benzene.

the intensity of the line of the unknown with that of another substance added in fixed amount to the unknown.^{153a}

6. Measurement of Depolarization Factor

The accurate measurement of the state of polarization of Raman lines is beset with some pitfalls, and requires a certain elaboration of equipment. The value of the depolarization factor depends upon the angle between the incident and scattered ray; and, if the incident beam is convergent, different rays will make different angles with their scattered rays. Errors thus caused can be partially eliminated by passing the exciting light through a

^{153a} D. H. Rank, R. W. Scott, and M. R. Fenske, *Ind. Eng. Chem., Anal. Ed.*, **14**, 816 (1942).

number of vertical slots¹⁵⁴ (Fig. 41). Another error to be guarded against is the different losses by reflection at prism and lens surfaces of the two components of linearly polarized light, the component with its electric vector vertical being more strongly reflected from the vertical prism faces than the horizontal component.

In principle, probably the best photographic way of measuring the state of polarization of Raman lines is to photograph the two components at once after resolution by a double-image prism or similar arrangement, and to measure the intensities by photographic photometry. If a Wollaston prism of large aperture is available, it can be conveniently placed in front of the camera lens, and the two spectra produced in juxtaposition¹⁵⁵ above and below each other can be photometered with the aid of a microdensitometer. Intensity marks are also photographed on the plate, to permit the density-intensity calibration to be effected. The intensity marks can be made by photographing the spectrum of an incandescent bulb of accurately known variable relative intensity (as by introducing a rotating sector, neutral screens, etc.), also resolved into perpendicular and parallel components by the double-image prism. The source being unpolarized, the correction to be applied at any wave length due to polarization introduced by the apparatus can also be found.

Polaroid has been extensively used for polarization measurements since its introduction for this purpose by Edsall and Wilson.¹⁴³ The literature on this use is summarized by Glockler and Baker,¹⁵⁶ who describe a method of simultaneously photographing the two components by placing two pieces of Polaroid polarizing filter in front of the slit, one transmitting the vertical component and the other the horizontal component of the scattered light.

Two mica half-wave plates are placed behind the Polaroids, one allowing the vibration entering it to pass unchanged, the other rotating the direction of the electric vector by 90° , so that the two beams fall on the prism vibrating in the same azimuth. In this way, if reflections before the beams enter the mica plates are avoided, the error caused by different reflection of the components at the prism surface is eliminated.

A 90° split field Polaroid disk may be used, placed in contact with the exit window of the Raman cell so that the upper half of the disk passes the vertical component, and the lower half passes the horizontal. The disk is imaged sharply at the slit of the spectrograph by a condensing lens, and the separated spectra due to the two components appear one above the other on the plate.

¹⁵⁴ A. Langseth, J. V. Sørensen, and J. R. Neilsen, *J. Chem. Phys.*, **2**, 402 (1934).

¹⁵⁵ W. West and R. B. Killingsworth, *J. Chem. Phys.*, **6**, 2 (1938).

¹⁵⁶ G. Glockler and H. T. Baker, *J. Chem. Phys.*, **11**, 446 (1943).

For qualitative purposes and, if the exciting light is sufficiently constant also for quantitative purposes, the state of polarization of Raman lines can be rather more easily determined by a procedure involving two successive exposures than by a single-exposure method. The simplest method, which readily distinguishes between strongly polarized and depolarized lines is that of Edsall and Wilson.¹⁴³ One exposure is made with the Raman tube surrounded by a Polaroid sheet formed into a coaxial tube oriented to transmit light whose electric vector vibrates parallel to the axis of the Raman tube. Completely polarized lines will therefore be absent from the light scattered perpendicularly to the direction of the incident radiation, and partially depolarized lines will have an intensity depending on the degree of depolarization. A second exposure is made with the polarizing tube removed, with the intensity of the exciting radiation reduced so as to make the density of the depolarized lines on the photographic plate equal to that of these lines in the first exposure. Lines of very different photographic density in the two exposures are therefore strongly polarized; those with about the same density are strongly depolarized.

The accuracy of this method is increased if both exposures are made by polarized light, the second, corresponding to the exposure by Edsall and Wilson with unpolarized light, by light transmitted by a Polaroid filter oriented to transmit the electric vector vibrating perpendicularly to the axis of the Raman tube.¹⁵⁷ If I_{\parallel} and I_{\perp} are the intensities of a Raman line for the respective exposures, the depolarization is I_{\parallel}/I_{\perp} . This method is used in the apparatus of figure 41. Two exposures for the same time are given, one through a polarizing filter made by wrapping a piece of Type H Polaroid (now replaced by Type K) around a glass tube with the optic axis parallel to the tube axis, the other through a similar filter with its optic axis at right angles to the tube axis.

TABLE X
DEPOLARIZATION FACTORS FOR CARBON TETRACHLORIDE

Raman line, cm. ⁻¹	Depolarization factor
218.....	0.857
314.....	0.857
459.....	0.046
762) 790)	0.857

In all work on polarization, it is well to check the apparatus by measuring the polarization of the carbon tetrachloride lines, which are easily photo-

¹⁵⁷ F. F. Cleveland and M. J. Murray, *J. Chem. Phys.*, **7**, 396 (1939).

graphed and whose polarization is well known. The values are given in Table X. The strongly polarized line at 459 cm.^{-1} represents the symmetrical valence vibration in which the four chlorine atoms move in phase back and forth along the bond direction.

VIII. PHOTOGRAPHIC PLATES FOR SPECTROSCOPY

1. Speed and Contrast

Photographic plates differ in speed, contrast, spectral sensitivity, graininess of the image, and resolving power. The speed and contrast are best described in terms of the characteristic curve of the plate, that is, the curve showing the relation between the optical density of the silver deposit and the exposure (intensity \times time) producing it. Hurter and Driffeld¹⁸⁸ first analyzed the behavior of plates in this way, plotting the density against the logarithm of the exposure, and the characteristic curves are often referred to as H and D curves. Figure 43 shows a typical form: A "toe" at low values of the exposure, in which the density increases only slowly with the exposure, followed often by a substantially linear portion, merging into a plateau in which increasing exposure is accompanied by no concomitant increase in density, which may be followed by a region in which the density actually falls with increasing exposure (solarization). The intercept of the linear portion of the curve with the axis of abscissae is the

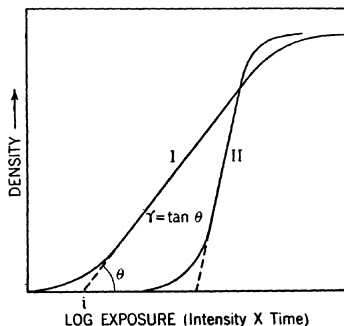


Fig. 43.—Characteristic curves of photographic plates: (I) a fast plate of low contrast; (II) a slow plate of higher contrast.

logarithm of an exposure i , called the inertia of the plate. The speed of the plate is defined as inversely proportional to i by the relation, speed =

¹⁸⁸ F. Hurter and V. C. Driffeld, *J. Soc. Chem. Ind.*, 9, 455 (1890).

10/i, the exposures being made by means of a standard source of specified intensity and spectral quality. Roughly speaking, the speed is a measure of the minimum exposure that will produce a visible image. The slope of the linear portion, denoted by γ , gives the increase in density for unit increase in the logarithm of the exposure, that is, it determines the contrast or gradation of the plate. γ depends on the time of development, increasing to a value γ_{∞} . As a general rule, high-speed plates have a low γ value and vice versa, though it is by no means true that of two plates that of the greater speed will necessarily have the lower γ value.

2. Reciprocity Failure

The speed of a plate, in general, depends on the intensity level of the illumination to which it is exposed. A given value of the product intensity \times time does not produce the same optical density, that is, the photographic effect is not determined simply by the total energy to which the plate is exposed. This is termed the failure of the reciprocity law, a failure common enough in photochemical reactions, as well as in the photographic process. At low intensities, the exposure, defined as the product of the intensity and time of illumination, required to produce a given photographic density may be several times that required at higher intensities. In photographing very feeble sources, such as faint fluorescence or Raman spectra, choice of a fast plate with small low-intensity reciprocity failure should be made. In calibrating plates for photometry, also, it is important that the calibrating intensities be of the same level as those to be measured.

3. Spectral Sensitivity

The ordinary photographic plate is sensitive to light of wave length shorter than about 5000 Å., but by appropriate sensitizers its sensitivity can be extended on the long-wave side to beyond 1.2 μ . The absorption of gelatin causes the sensitivity to fall off rapidly in the ultraviolet at wave lengths shorter than about 2500 Å. Plates, however, can be sensitized to this region by gently swabbing the emulsion with fluorescent material, e. g., Nujol, transformer oil, etc., before exposure, care being taken to remove the oil by bathing the exposed plate in acetone before developing. The short ultraviolet is absorbed by the oil, which re-emits the radiation as fluorescent light of longer wave lengths than those absorbed by the gelatin. In the use of fluorescent sensitizers for the short ultraviolet, it is necessary to avoid mistaking absorption regions of the sensitizer for absorption of the material under study. Plates sensitized for the short ultraviolet are available from *Eastman Kodak Co.*

Schumann's work in the vacuum ultraviolet was carried out by means of plates prepared by himself containing a very small amount of gelatin.

Such plates are obtainable from *Ilford Co.*, Ilford, England, and from *Adam Hilger, Ltd.* The ultraviolet-sensitized plates of *Eastman Kodak Co.* can also be used in the Schumann region.

Commercial plates comprise a large range of sensitometric and spectral qualities as well as of graininess and resolution; for details, reference is made to the booklet, "Photographic Plates for Use in Spectroscopy and Astronomy," supplied by *Eastman Kodak Co.*, Rochester, N. Y. This booklet also contains a very useful account of the essentials of the practice of spectroscopic photography.

Absorption spectrography in the organic laboratory will not usually be troubled by low intensity in the source. The best plates to use in these circumstances are those of moderate contrast and speed, and as low graininess as possible. Lack of graininess is particularly important when the plates are to be microdensitometered. Kodak Spectroscopic Plates type III are suitable. In recording feeble emission spectra, speed is the first requirement, and the best compromise on contrast and graininess must be made. Kodak Type 103a is suitable at low levels of intensity, and Type 103 is a relatively fast plate of unusually low granularity for a plate of its speed.

Uniformity of development is important for spectroscopic purposes, particularly in photometry. Development in an ordinary tray by brushing with a fine, broad, camel's-hair brush is an easy way of securing considerable uniformity; and placing the plate in a mount so that, always completely immersed in developer, it is moved fairly rapidly in a vertical plane in the solution also gives good results. The processing solutions should be fresh and at the temperature recommended by the manufacturer. It is self-evident also that the dark room should be kept clean; developing and fixing solutions should not be allowed to evaporate in trays, and drops of solution should not be allowed to dry on the floor or bench—otherwise dust particles of the chemicals falling on plates, etc. can produce erratic results.

IX. SOME APPLICATIONS OF SPECTROSCOPY TO ORGANIC CHEMISTRY

1. Chemical Analysis^{158a}

This section is intended merely to outline some important applications of spectra to organic chemistry. The applications may be summarized

^{158a} A complete survey of recent instrumental developments and applications of spectroscopic methods in chemical analysis is made in the articles on "Light Absorption Spectrometry," by M. G. Mellon, "Infrared Spectroscopy," by R. B. Barnes and R. C. Gore, "Raman Spectra," by W. G. Braun and M. R. Fenske, and "Ultraviolet Absorption Spectrophotometry," by E. J. Rosenbaum, in "The First Annual Review of Analytical Chemistry," *Anal. Chem.*, **21**, 3, 7, 12, 16 (1949).

into two classes, which are sometimes not strictly separable: (1) those involving qualitative and quantitative analysis by empirical comparison with the spectra of known substances; and (2) those in which a more or less complete structural determination of a compound is attempted from a knowledge of its spectrum. The prime fact for analysis is that every compound has a specific spectrum in some region of the infrared, visible, or ultraviolet region which may offer a convenient and highly sensitive means of detection and estimation. One of the simplest and most useful applications is the determination of impurities, which is very easily performed if the impurity has absorption bands in regions to which the main component is highly transparent. For example, paraffins and saturated aliphatic alcohols, ethers, and acids absorb only slightly throughout most of the quartz ultraviolet, and any absorption in the longer regions of the ultraviolet indicates the presence of an impurity. Small amounts of benzene in ethyl alcohol, as are present in material prepared by azeotropic distillation, can be detected by the appearance of the characteristic band of benzene absorption at about 2600 Å. (see Fig. 1a); acetaldehyde in alcohol or acetone in acetic acid are similarly readily detected by the carbonyl absorption band at about 2800 Å. (see Fig. 1c). Very small amounts of water in many organic compounds can be detected by means of its absorption band at about 3 μ , for example, 0.01% of water in dioxane can be found in a thickness of a few mm. and the sensitivity probably could be increased by increasing the cell thickness. Similarly, phosgene in chloroform can be detected by its strong absorption band at 5.49 μ and thiophene in benzene by its absorption at 7.09 μ .

It has already been pointed out (page 1336) that the infrared spectrum of an organic compound can be roughly divided into two regions: (1) the shorter region, of wave length less than about 7 μ , in which the strongest bands are associated with various types of valence linkages in the molecule such as C—H, C=O, C \equiv N, and so on, and do not differ greatly in frequency from compound to compound containing the relevant linkage; and (2) the longer region which, in addition to containing some of the lower "bond frequencies," contains bands which, originating in the vibration of relatively large parts of the molecule with respect to each other, may be highly characteristic and specific for the individual molecule. Examples are contained in figure 28, showing the distinction between 1,3-dimethyl- and 1,1-dimethylbutadiene, and in figure 35 for 2,2,4- and 2,3,4-trimethylpentane. Infrared methods are especially important in the qualitative and quantitative analysis of saturated hydrocarbons, whose ultraviolet absorption spectra occur only in the Schumann region. Moreover, these ultraviolet spectra are unsuitable for analysis because of overlapping continua. A recent application, which illustrates the power of infrared ab-

sorption methods in the analysis of small quantities of substances difficult to characterize uniquely by other methods, is the identification of phthiocerane, the hydrocarbon from the wax alcohol phthiocerol of the tubercle bacillus as 4-methyltritriacontane.¹⁵⁹ The spectrum of phthiocerane was found to be identical with that of 4-methyltritriacontane and 4-methyl-tetratriacontane, but markedly different from those of the 2-, 3-, and 5-methyl-substituted compounds, the 4-methyl compounds having a characteristic band at 740 cm^{-1} . Added to x-ray and melting-point data, the infrared spectrum is considered to permit a conclusive identification of phthiocerane with 4-methyltritriacontane. 1 mg. of the hydrocarbon was used in the infrared study, which presumably was recoverable.

A prerequisite for quantitative analysis of a mixture by spectral absorption methods is a knowledge of the spectra of all of the pure components. The observed spectrum of the mixture must be accounted for completely in terms of bands of the pure components. If a band is present which cannot be identified, an unknown component is present which may contribute in an unknown fashion to any of the bands selected for measurement.

As in other applications of analytical chemistry, no fixed rules of procedure can be given to govern all cases of estimation by absorption spectrophotometry, and much must be left to the discretion of the analyst according to the circumstances. For example, the analysis of a complicated mixture may be speeded up and made more accurate if the number of components subjected to a given procedure can be reduced. If, for instance, one component can be readily removed and estimated by distillation, and another readily analyzed by ultraviolet spectrophotometry, the analysis of the remaining components by infrared methods would probably be made with considerably greater accuracy and speed than otherwise, and the subsequent computational labor lightened. The general principle in analytical spectrophotometry in any spectral region is to select an intense band for each component in a spectral region where all the other components absorb feebly. Accuracy is highest for a peak absorption of about 63% but it may prove advantageous to decrease this figure somewhat if this causes less overlapping with the bands of other components.

The estimation of the composition of a binary mixture is made from a working curve of transmission or density of the component of interest, at a wave length at which it alone absorbs strongly, as a function of its concentration in standard mixtures of the two components. A purely empirical transmission curve may be used without making corrections for absorption and reflection by the cell windows, or for spurious radiation (page 1328), or for absorption by the other component, which will remain valid so long

¹⁵⁹ S. Stöhlberg-Stenhagen, E. Stenhagen, N. Sheppard, G. B. B. M. Sutherland, and A. Walsh, *Nature*, **166**, 580 (1947).

as these quantities and the cell thickness remain unaltered. It is advisable to check this curve at intervals, as the deterioration of rock-salt windows may change both the transmittance of the windows and the thickness of the absorbing layer. The other procedure is to make corrections for the transmission factors for windows and solvent, or, as in double-beam procedures, to compensate for these factors automatically, and to correct for spurious radiation. As the working curve, a plot is made of the true density for the component in the mixture as a function of its concentration. If Beer's law is obeyed, the curve will be linear, passing through the origin. If this law has once been established for a system, re-checking of the working curve requires only two points to be established.

Multicomponent systems can be analyzed computationally from the observed absorption of the mixture at a number of suitable wave lengths if the components absorb independently and the extinction coefficient of each component is known. The measured density at any wave length is then:

$$D = (\epsilon_1 c_1 + \epsilon_2 c_2 + \dots)d + K \quad (26)$$

where the ϵ 's are the extinction coefficients of the various compounds at the given wave length, the c 's the corresponding concentrations, d the thickness of the absorbing layer, and K the "cell constant,"¹⁵⁰ *i. e.*, the contribution of the windows to the measured density. In double-beam arrangements with matched cells, K is compensated for and does not enter the equation. If n components are to be analyzed for, measurements at n different wave lengths are made, and the system of n simultaneous equations of the form of equation (26) solved for the c 's. K is a slowly varying function of the wave length and can be determined by measuring the density of the cell filled with a practically transparent material for the wave length in question, or by calibrating the cell with a substance of known extinction coefficient in a transparent solvent. The solution for two absorbing components is given on page 1430 of Chapter XXII. Computing devices for solving the simultaneous equations, which becomes very laborious when the number of components increases, are described by Stewart in Chapter XXXI in connection with the similar problem of analysis by mass spectroscopy.

Nielsen and Smith¹⁶⁰ have shown that sensitivity of the analysis of a given component is greatest when it has an intense band at a wave length at which the other components show little absorption. They describe a simplified procedure for dealing with a ternary mixture with two minor components. A wave length, λ_1 , is chosen at which the minor component 1 has a strong band and the others absorb feebly. A density-concentration curve is constructed for component 1 in the major component, the unit of

¹⁶⁰ J. R. Nielsen and D. C. Smith, *J. Ind. Eng. Chem.*, 15, 609 (1943).

concentration being percentage by weight or volume. The observed density of the ternary mixture at λ_1 is then used to obtain the approximate concentration \bar{c}_1 of 1 from the curve for the binary system 1 in the major component. Similarly, wave length λ_2 is selected at which the component 2 alone absorbs strongly and the approximate concentration \bar{c}_2 is read off from the corresponding working curve for the binary system 2 in the major component using the observed density of the ternary mixture at λ_2 . The corrected concentrations are then computed from the following equations:

$$c_1 = \bar{c}_1 - \frac{(\epsilon_2)_1 - (\epsilon_0)_1}{(\epsilon_1)_1 - (\epsilon_0)_1} \cdot \bar{c}_2 \quad (27)$$

$$c_2 = \bar{c}_2 - \frac{(\epsilon_1)_2 - (\epsilon_0)_2}{(\epsilon_2)_2 - (\epsilon_0)_2} \cdot \bar{c}_1 \quad (28)$$

where $(\epsilon_0)_1$, $(\epsilon_1)_1$, and $(\epsilon_2)_1$ are the extinction coefficients for the major component and the minor components 1 and 2, respectively at λ_1 , and $(\epsilon_0)_2$, etc., the corresponding extinction coefficients at λ_2 . These equations, for whose derivation reference is made to the article by Nielsen and Smith,¹⁶⁰ are themselves approximate, but the error introduced is not more than about 0.1% of the measured concentration, provided that the components absorb independently and that proper corrections for the cell constant and for spurious radiation have been made.

Examples of simplified special methods of chemical analysis by infrared absorption are furnished by Barnes, Liddel, and Williams,¹⁶¹ and by Wright.¹⁶² The infrared analysis of isotopic mixtures of deuteromethanes and ethanes is described by Benedict, Marikawa, Barnes, and Taylor.¹⁶³ In the ultraviolet an example is the determination of benzene vapor in toluene vapor in the range of 0.01 to 10%.¹⁶⁴

Raman spectra are not, in general, sensitive to small quantities, but can be useful in special cases. For example, 1% of *m*-xylene in an *o*-*p*-mixture can be detected. The use of Raman spectra for analysis is discussed by Goubeau and Thaler,¹⁶⁵ by Landsberg and coworkers,¹⁶⁶ and recently by Braun and Fenske.^{167a}

The spectroscopic determination of concentrations of substances in

¹⁶¹ R. B. Barnes, V. Liddel, and V. Z. Williams, *Ind. Eng. Chem., Anal. Ed.*, **15**, 659 (1943).

¹⁶² W. Wright, *ibid.*, **13**, 1 (1941).

¹⁶³ W. S. Benedict, K. Marikawa, R. B. Barnes, and H. S. Taylor, *J. Chem. Phys.*, **5**, 1 (1937).

¹⁶⁴ P. A. Cole and D. W. Armstrong, *J. Optical Soc. Am.*, **31**, 740 (1941).

¹⁶⁵ J. Goubeau and L. Thaler, *Angew. Chem.*, **54**, 26 (1941).

¹⁶⁶ G. S. Landsberg, P. A. Bazhulin, Y. V. Rosenberg, and A. S. Elenev, *Bull. acad. sci. U.R.S.S.*, **4**, 158 (1940).

equilibrium can, of course, lead to the determination of the thermodynamic characteristics of the equilibrium, according to the well-known relations between the concentrations of reactants and resultants, temperature, equilibrium constant, free energy change, and heat of reaction. An example is the spectroscopic determination of the heat of dimerization of acetic acid in the vapor phase.¹⁶⁷ Sometimes empirical relations between spectroscopic quantities and thermal quantities emerge, as in the Badger and Bauer relation between the shift of frequency produced when hydrogen bonds are formed and the energy of the bond.¹⁶⁸

2. Determination of Structure

There are various levels of completeness at which the structure of organic molecules can be determined with the aid of spectroscopic information. The most complete description, which can furnish detailed information in favorable cases on the shape of the molecule and the interatomic distances, is achieved by the method most limited in scope. Only by a thorough analysis of the rotational structure of the absorption bands of gases (Fig. 27*a*, *b*) is it possible to make a direct spectroscopic determination of interatomic distances. The specialized techniques of the highest spectroscopic resolution and of the theoretical analysis of the observations are scarcely within the province of the organic chemist. In any case, only a few simple molecules of high symmetry are at all amenable to this treatment, and by far the greatest number of organic molecules seem doomed to remain untouched by these methods.

A somewhat lower level of structure determination, leading to a knowledge of the shape of molecules of adequate symmetry, depends on the analysis of the vibrational spectrum, as shown by infrared and Raman methods. The conclusions are most certain when the spectra of the substance in the gaseous state are known. Only moderate resolution is required, similar to that of most of the instruments described in this chapter. The chief theoretical requirement is a knowledge of the selection rules which determine the intensity of the various vibrational bands in their dependence on molecular symmetry, for which reference to the various treatises on Raman and infrared spectroscopy is made. Again, molecules of relatively high symmetry are most amenable to this treatment, which often involves a study of the effects of isotopic substitutions. On this level, spectroscopic methods have made some notable contributions to fundamental problems of structural organic chemistry. Probably the most important example is the use by Ingold and his collaborators of most of

¹⁶⁷ R. C. Herman and R. Hofstadter, *J. Chem. Phys.*, **7**, 460 (1939).

¹⁶⁸ R. M. Badger and S. H. Bauer, *J. Chem. Phys.*, **5**, 839 (1937).

the resources of spectroscopy, infrared and ultraviolet absorption, and Raman and fluorescence spectra in the study of the structure of the benzene molecule.¹⁶⁹

Although no exact determination of interatomic distances can be made without a knowledge of rotational structure, useful estimates are possible, based on empirical or semiempirical relations between the force constants associated with the vibrations (page 1333) and the internuclear distances. The equation of Badger:¹⁷⁰

$$k = c_{ij}/(r_e - d_{ij})^3 \quad (29)$$

is representative of the type. Here k is the bond-stretching force constant, r_e the distance between the bonded atoms at the equilibrium position, and c_{ij} , d_{ij} are parameters which depend on the position of the atoms in the periodic table.

The least complete determination of structure from spectral data, but that most immediately and widely applicable to the practical problems of the organic chemist, is the partial structural identification of a compound depending on the presence or absence of certain bands in the spectrum. For this application, the liquid as well as the gaseous phase can be used. The existence of characteristic infrared and Raman frequencies which can be identified with the presence of definite linkages in the molecule has already been referred to (page 1336). Table XI lists some of these "bond

TABLE XI
INFRARED AND RAMAN BOND FREQUENCIES

Group	Frequency, cm. ⁻¹	Group	Frequency, cm. ⁻¹
C—H with saturated C atoms	2700-3000	C=C	1620-1670
C—H in aromatic compounds	3000-3100	C=O aldehydes	1720
C—H in olefins	3000-3100	C=O acids ^b	1650
C—H in acetylenic compounds	3300	C=N as in acetaldazine	1630
N—H in R—NH ₂	3300-3370	N=N as in azomethane	1440
O—H in R—OH ^a	3400	C≡C	2100-2230
S—H in R—SH	2575	C≡N nitriles	2245
Se—H in R—SeH	2300	N≡N nitrogen molecule	2360
C—C in open chains	750-1100	NO ₂ in nitro compounds	1380, 1550
C—O	ca. 1000	C—Cl	600-700
N—N	750-1100	C—Br	560
		C—I	500

^a This is the value found in liquid alcohols or in concentrated solutions in carbon tetrachloride, and is due to O—H linked to other molecules by hydrogen bonds. In the vapor or in dilute solution, a higher value about 3600 cm. ⁻¹ is found for the free O—H vibration.

^b See table XII for values of the C=O frequency in other molecules.

¹⁶⁹ C. K. Ingold, *Proc. Roy. Soc. London*, **A169**, 148 (1938).

¹⁷⁰ R. M. Badger, *J. Chem. Phys.*, **2**, 128 (1934); **3**, 710 (1935). See W. Gordy, *ibid.*, **14**, 305 (1946).

frequencies." For example, a molecule known to contain sulfur which shows strong infrared and Raman frequencies at about 2575 cm^{-1} contains the S—H linkage; and, indeed, if the substance were completely unknown, the appearance of this frequency would strongly suggest S—H. As a simple example of the application of a knowledge of bond frequencies to a structural problem, consider the structure of the nitriles and the isocyanides. Acetonitrile has strong infrared and Raman frequencies at 2250 cm^{-1} , and methyl isocyanide at 2165 cm^{-1} . In the nitrile there is no doubt that the frequency 2250 cm^{-1} is to be ascribed to the $\text{C}\equiv\text{N}$ linkage. If the isocyanide contained carbon linked to a nitrogen atom by a double bond, as in the older formulation, the frequency would be in the region 1600 to 1700 cm^{-1} , where those of $\text{C}=\text{C}$, $\text{C}=\text{O}$, and $\text{C}=\text{N}$ are known to occur (Table XI). The carbon-to-nitrogen linkage in the isocyanides must be essentially a triple bond, $\text{CH}_3-\text{N}^+\equiv\text{C}^-$. At the same time, the frequency of the isocyanides is sufficiently lower than that of the nitriles to permit discrimination between the two groups of compounds.

This illustrates another important feature of the bond frequencies of great value in the study of structure—they are subject to definite influences of their intramolecular environment. For instance, a large number of compounds containing the carbonyl group have infrared absorption and Raman frequencies between about 1650 and 1790 cm^{-1} . The frequencies of a particular type of $\text{C}=\text{O}$, say the aldehydic, are grouped about a much narrower range, and it is possible to compile a table of $\text{C}=\text{O}$ frequencies in the various types of compounds. In table XII, R is an alkyl radical. The acids and amides have carbonyl frequencies about 50 or 60 cm^{-1} lower than the aldehydes and ketones; the esters have appreciably higher frequencies; and the acid chlorides have abnormally high frequencies. In ethyl acetate, two frequencies in this region are found, at 1714 and 1740 cm^{-1} , which, no doubt, represent the ketonic and estereal carbonyl, respectively.

TABLE XII
INFRARED AND RAMAN CARBONYL FREQUENCIES

Group	Structure	Frequency, cm^{-1}
Aldehyde	$\text{R}\cdot\text{CO}\cdot\text{H}$	1780
Ketone	$\text{R}\cdot\text{CO}\cdot\text{R}$	1710
Acid	$\text{R}\cdot\text{CO}\cdot\text{OH}$	1656
Amide	$\text{R}\cdot\text{CO}\cdot\text{NH}_2$	1662
Ester	$\text{R}\cdot\text{CO}\cdot\text{OR}$	1735
Acid chloride	$\text{R}\cdot\text{CO}\cdot\text{Cl}$	1792

Another important example of environmental influence on bond frequencies is the effect of an adjacent double bond on a frequency. Table XI shows how the frequencies of the C—H vibration increase with the un-

saturated character of the carbon atoms to which they are linked. The phenomenon appears to occur with certain other linkages; for example, the Raman spectra of olefinic and aromatic chloro compounds show higher frequencies, that appear to be associated with the C—Cl vibrations, than are present in corresponding saturated compounds. In this case the higher frequencies may be associated with resonance between the normal structure and one involving doubly bonded halogen¹⁷¹ and the increase in the C—H frequency in unsaturated compounds with hyperconjugation. As has been frequently mentioned in Section VI, the infrared spectra of organic compounds, besides exhibiting frequencies characteristic of specific bonds, also show (mostly in the longer wave-length region) frequencies associated with the motion of complex groupings within the molecule which often constitute the “key bands” in the analysis of mixtures of similar compounds. Also the “bending vibrations” of hydrogen atoms (page 1334) may be sensitive to structure. For example, the deformational frequency of the methylene group is about 1460 cm.⁻¹ and the symmetrical deformation frequency of the methyl group is about 1375 cm.⁻¹ (cf. Fig. 26, ν_3).¹⁷² It is found that the polymerization product from ethylene, polythene, which was first supposed to contain an uninterrupted chain of methylene groups, shows a band at 1375 cm.⁻¹ in addition to a stronger band at 1460 cm.⁻¹, and the conclusion is drawn, in confirmation of earlier evidence derived from a study of the C—H stretching vibrations about 3 μ ,¹⁷³ that methyl groups are present in the polymer. From studies of the intensity it is estimated that one methyl group is present for about every 50 methylene groups.

Another example is the frequency of the deformational vibration in olefinic compounds in which the hydrogen atoms move out of the plane of the ethylenic carbon atoms. In ethylene this frequency is at 951 cm.⁻¹ and substituted ethylenes have very strong bands in this region whose number and position depend on the substituent.¹⁷⁴ It is possible in this way to differentiate between $\text{CHR}=\text{CH}_2$, $\text{CHR}=\text{CHR}$, $\text{CR}_2=\text{CH}_2$, and $\text{CHR}=\text{CR}_2$.

The application of these facts is rather obvious. Characterization of unknown compounds can be facilitated, the course of reactions followed, the disappearance seen of reacting bonds, and the appearance of new ones. For example, Gantmacher and Medvedev¹⁷⁵ observed by Raman spectra, among other changes in the polymerization of chloroprene and isoprene, the weakening of the intense frequency, 1620 cm.⁻¹, attributed to the con-

¹⁷¹ L. Pauling, *The Nature of the Chemical Bond*. 2nd ed., Cornell Univ. Press, Ithaca, 1940, p. 214.

¹⁷² H. W. Thompson and P. Torkington, *Trans. Faraday Soc.*, **51**, 246 (1945).

¹⁷³ J. J. Fox and A. E. Martin, *Proc. Roy. Soc. London*, **A175**, 208 (1940).

¹⁷⁴ R. S. Rasmussen and R. R. Brattain, *J. Chem. Phys.*, **15**, 120 (1947).

¹⁷⁵ A. R. Gantmacher and S. S. Medvedev, *Rubber Chem. Tech.*, **16**, 841 (1943).

jugated double-bond system of the monomer, and the appearance, with increasing intensity, of the frequency 1660 cm.^{-1} of the isolated double bond. Similar observations in other polymerization reactions have been made in the infrared.

3. Choice of Raman or Infrared Measurements

Both Raman and infrared absorption spectra give, within the limitations of the selection rules governing the intensity of the transitions in the two phenomena, essentially the same information about the molecule, its vibration spectrum in the normal electronic state. If the practical problem is a mere matter of characterization of a pure substance or of a mixture containing substantial amounts of all the components, there is probably little in principle to choose between the two methods, and the choice will be determined by convenience and the availability of equipment. To set up a Raman apparatus is less costly in time and money than to set up an infrared spectrometer; and, where only occasional work is contemplated and the demands on sensitivity to small quantities are not high, the Raman equipment would probably be adequate if visual illumination of the sample is permissible. But where sensitivity is required, or where a prolonged analytical program covering a larger variety of substances is envisaged, the greater power and adaptability of infrared equipment would probably give it preference. Nevertheless, as automatic photoelectric recording becomes perfected, it may well turn out that Raman spectroscopy will compete, as an analytical tool, for suitable systems, on equal terms with infrared and ultraviolet spectroscopy. For analytical procedures by means of Raman spectroscopy, see Stamm^{175a} and others.^{158a}

Where the prime interest is in detailed structural analysis of organic molecules, the theory of the phenomena shows that the two methods are not alternatives, but supplementary to each other.

4. Ultraviolet Spectra

In certain cases ultraviolet spectra show vibrational structure, but the strongest bands in absorption are associated with vibrations in the excited electronic state, which usually differ in frequency from the value in the normal state. The determination of the frequencies of these vibrations in electronically excited states is made from measurements of the frequency interval between the vibrational bands and, since the observed spectra of liquids or solutions may contain, in an apparently single band, several unresolved vibrational bands (see Fig. 1a), conclusions are most certain from observations of gases at high resolution. In fluorescence, the vibrational

^{175a} R. F. Stamm, *Ind. Eng. Chem., Anal. Ed.*, **17**, 318 (1945).

intervals in the spectrum correspond to frequencies in the normal electronic state. More important in the ultraviolet for characterizing compounds than the bond frequencies are the positions of the electronic band system and the nature of the spectrum, whether it is continuous or shows well-defined vibrational structure. For instance, the aliphatic aldehydes and ketones have a very characteristic, usually structureless, absorption spectrum with a maximum about 2800 Å., of relatively low molecular extinction coefficient. This is a criterion for the presence of an aldehydic or ketonic carbonyl group in these compounds. In the same way, the alkyl iodides have a characteristic continuous absorption with a maximum about 2500 Å. Other characteristic ultraviolet "group spectra" are C—Br about 2000 Å., C—Cl about 1750 Å., C=S about 3300 Å., N=N about 3700 Å.

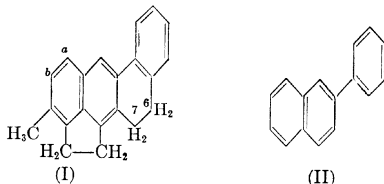
These "electronic group spectra" appear to be subject to greater environmental influence than the characteristic infrared bond vibrations, *e. g.*, in crotonaldehyde, $\text{CH}_3\text{CH}=\text{CHCHO}$, the band appears at 3100 Å. with some increase in intensity; in acrolein, $\text{CH}_2=\text{CHCHO}$, at 3400 Å., with the appearance of vibrational structure; and in glyoxal, $\text{O}=\text{CHCH}=\text{O}$, the shift is so great that the band enters the visible.

The association of C=O with OH, OR, NH_2 , and Cl produces profound changes in the ultraviolet spectrum. The aliphatic acids, amides, and esters are quite transparent at 2800 Å. Only at about 2100 Å. do the aliphatic acids show a band which may represent the carbonyl in carboxyl. The acid chlorides have a band at 2400 Å. In the amides, a band at 2100 Å. is overlapped by a more intense one at shorter wave lengths, so that in the quartz spectrograph they show only "end absorption," that is, a continuous increase in the absorption toward the short-wave end of the plate.

Conjugation tends to be accompanied by the appearance in the ultraviolet spectra of more or less distinct vibrational structure, which becomes particularly well marked in the aromatic hydrocarbons (Fig. 1a, page 1246). The appearance of such a spectrum at about 2700 Å. or longer suggests immediately the existence in the system of one or more aromatic nuclei. It is to be noted, though, that the introduction of certain substituents greatly reduces this characteristic of aromatic spectra (Fig. 1b). The wave length of the longest absorption band is displaced to lower values as more rings are condensed, as shown by the series benzene, naphthalene, anthracene, naphthacene, for which the wave lengths of the longest strong band are, respectively, about 2700, 3150, 3750, and 5098 Å. On the other hand, the spectrum of biphenyl resembles that of benzene. As an interesting example of a structural identification made through a consideration of the relation between ring structure and ultraviolet absorption spectra may be mentioned the identification¹⁷⁶ by Fieser and Hershberg of a di-

¹⁷⁶ L. F. Fieser and E. B. Hershberg, *J. Am. Chem. Soc.*, **60**, 940 (1938).

hydromethylcholanthrene as the 6,7-dihydro derivative (I) on the basis of the resemblance of its spectrum to that of β -phenylnaphthalene (II). Reduction at the a and b positions of (I) would have given a compound



whose spectrum resembled that of phenanthrene, which is similar to that of anthracene, with lower intensity. The very considerable detail with which the spectroscopic differentiation of quite similar polynuclear hydrocarbons can be accomplished is illustrated in the work of Jones.¹⁷⁷

Finally, reference may be made to the light which can be thrown on details of chemical behavior by the analysis of empirical spectral data in terms of modern concepts of organic chemistry such as that of resonance. A case in point is the work of Brooker¹⁷⁸ and coworkers on the color and constitution of certain dyes. Not only has order been brought into a vast welter of formerly uncoordinated data on the absorption spectra of these dyes, but information has emerged on matters of chemical importance, such as the relative basic strengths of heterocyclic nuclei in dye molecules.

A development in spectrostructural analysis of interest to organic chemists is the recognition of steric hindrance effects on ultraviolet absorption spectra. From the fundamental nature of the electronic linkage in the double bond, it follows that if a spectrum is due essentially to the existence of resonating structures in a molecule, its position will be changed and its intensity reduced if the resonating chain is prevented from assuming a planar configuration by neighboring groups impinging on it. The existence of this effect should be borne in mind in attempting structural identification from "group bands" in the ultraviolet; for example, *o*-nitro-*tert*-butylbenzene and *o*-nitrocumene have almost lost the strong structureless absorption band at about 2500 to 2600 Å. characteristic of nitrated benzene derivatives, while the corresponding para compounds show the bands very strongly.¹⁷⁹ Other examples of steric hindrance on absorption spectra are given by Brooker (cyanine dyes),¹⁸⁰ Jones (ring hydrocar-

¹⁷⁷ R. N. Jones, *J. Am. Chem. Soc.*, **63**, 151, 313 (1941).

¹⁷⁸ L. G. S. Brooker, *Rev. Modern Phys.*, **14**, 275 (1942).

¹⁷⁹ W. G. Brown and H. Reagan, *J. Am. Chem. Soc.*, **69**, 1033 (1947).

¹⁸⁰ L. G. S. Brooker, *Chem. Revs.*, **41**, 325 (1947).

bons),¹⁸¹ Remington (nitrocompounds),¹⁸² Rodebush and coworkers (biphenyls),^{183, 184} Sherwood and Calvin (nitro compounds),¹⁸⁵ and others. The correlation of these effects with chemical reactivity may offer some interesting results.

¹⁸¹ R. N. Jones, *J. Am. Chem. Soc.*, **63**, 1658 (1941).

¹⁸² W. R. Remington, *ibid.*, **67**, 1838 (1945).

¹⁸³ M. T. O'Shaughnessy and W. H. Rodebush, *ibid.*, **62**, 2906 (1940).

¹⁸⁴ B. Williamson and W. H. Rodebush, *ibid.*, **63**, 3018 (1941).

¹⁸⁵ D. W. Sherwood and M. M. Calvin, *ibid.*, **64**, 1350 (1942).

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I. INTRODUCTION

The subject of this section is the instrumental procedure in chemical analysis depending on: (a) comparative observations of the intensity of color developed in a system which absorbs visible light; or (b) an absolute measurement of the amount of light absorbed by the system. The procedures in the first of these categories comprise the long-established "colorimetry" of chemists, a misnamed term sanctioned by long and well-understood chemical usage as a designation for chemical analysis depending on the *comparison* of the color of the substance under investigation with that of a standard. An objective equivalent to this subjective procedure is also possible, with the substitution of photoelectric cells for the eye. Recent emphasis, however, has been on the development of rapid methods of *measurement* of the light absorbed by the analytical system, that is, of photometric methods. The great advantage of such measurements over

the older methods of comparison is the avoidance of the use of standards, after a preliminary calibration has been made, and often there is a very considerable gain in sensitivity. Both subjective and objective methods of photometry are employed, as represented, for instance, by the use of the Pulfrich photometer and the host of photoelectric photometers, respectively.

Photometric chemical analysis is most advantageously carried out with light contained within a relatively narrow band of wave lengths, which for many purposes is adequately furnished by filters, that is, by materials in the form of plates of colored glass, gelatin films, absorbing solutions, etc., transmitting only a restricted spectral region. Photometers permitting measurements in wave-length regions defined by filters can be appropriately termed *filter photometers*. There has been some disposition shown in the literature to describe the operation of a filter photometer as that of an "abridged spectrophotometer." Absorption spectrophotometry, however (page 1295), is the operation of mapping the intensity of absorption in a spectrum at wave-length intervals arbitrarily small and over bands arbitrarily narrow. If this could be done by filter photometers, there would be no objection to describing their use as spectrophotometry, but, as this is not so with instruments available at present, it seems desirable to denote them by means of the less pretentious name.

In addition to the methods of analysis depending on the absorption of light, it is possible to utilize the emission of light by fluorescent substances. Over a restricted range of concentrations, the intensity of fluorescence is nearly proportional to the concentration, and by slight modification of the instruments used for absorption photometry it is possible to measure the intensity of fluorescent light with precision. A short section has therefore been added on the principles and methods of fluorimetry (see pages 1439-1448).

II. THEORY

1. Beer's Law

The laws of the absorption of light have been discussed in detail in Chapter XXI, page 1295, and we shall merely state here Beer's law for the absorption of a monochromatic parallel beam of wave length λ , incident intensity I_0 , and emergent intensity I_λ , by a solution of a single substance of concentration c moles per liter and layer thickness d centimeters in a transparent solvent:

$$\log_{10} I_0/I_\lambda = D_\lambda = \epsilon_\lambda cd \quad (1)$$

The quantity D is called the density of the transmitted radiation (page 1296), or sometimes the extinction. The molar extinction coefficient, ϵ_λ , is

independent of the concentration at a given temperature and in a given medium, but varies with wave length, temperature, and medium. If Beer's law is true, the density at constant thickness of the absorbing layer is seen to be proportional to the concentration of absorbing substance.

Usually the absorption spectra of solutions consist of broad bands covering an extended wave-length region with variable molar extinction coefficient (see Fig. 1). If light containing wave lengths for which the extinction coefficients are markedly different is incident on a layer of constant thickness of solution, the density of the total transmitted radiation is not

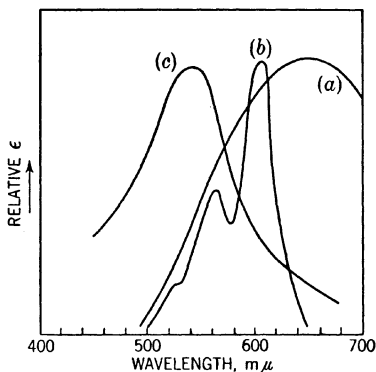


Fig. 1.—Some types of absorption band in solution: (a) copper sulfate¹ in large excess of ammonium hydroxide; (b) pinacyanol² in ethyl alcohol, $\epsilon_{\max.} = 1.95 \times 10^4$; (c) chromium diphenylcarbazide complex,³ $\epsilon_{\max.} = 8.14 \times 10^4$.

proportional to the concentration.* Equation (1) is valid strictly only for monochromatic light, and with heterochromatic light to a more or less high degree of approximation the more or less nearly uniform the value of the extinction coefficient over the wave-length band. Provided they inherently conform to Beer's law, solutions with flat-topped absorption curves, as in figure 1, curve a, will therefore "obey Beer's law" over a wider band of heterochromatic radiation within the absorption band than those with sharp maxima, as in curve b.

2. Principle of Balancing

The logarithmic expression (1) implies that each molecule of the absorbing substance at constant temperature and in a constant environment

¹ F. Weigert, *Ber.*, **49**, 1530 (1916).

² S. E. Sheppard, *Rev. Modern Phys.*, **14**, 312 (1942).

³ G. P. Rowland, Jr., *Ind. Eng. Chem., Anal. Ed.*, **11**, 442 (1939).

absorbs the same fraction of the light incident on it. If, therefore, a solution has a single absorption band in the visible, for which Beer's law is true at all wave lengths, then the color seen by transmission of an incident beam of white light through a column of the medium will depend only on the total number of absorbing molecules in the length of the column. Hence, if beams of equal incident intensity of white light are passed through two columns of such an absorbing solution containing different concentrations of the absorbing substance, the intensities of the transmitted beams will be judged equal when the column lengths are inversely proportional to the concentrations. This is the basis of the well-known "balancing method" of colorimetry, in which, by appropriate devices, the two beams are brought together to facilitate comparison, and the lengths of the absorbing columns, one of sample, the other of known concentration of the same absorbing medium, are adjusted until the beams match or "balance." At balance, the condition *concentration* \times *thickness* = *constant* prevails, or if the sample and standard are designated by subscripts *x* and *s*:

$$c_x d_x = c_s d_s \quad (2)$$

where *c* and *d* refer to concentration and column thickness, respectively.

3. Absolute Photometry

Simple and precise as the method of subjective balancing can be made, modern developments in photometry have led to the devising of methods of colorimetric analysis which threaten largely to displace the older methods. By means of a photometer, an absolute determination of the light transmitted by the sample can be made. If Beer's law is true for the substance, the concentration can be expressed as a function of the light transmitted, by transposing equation (1) to the form:

$$c = 1/\epsilon d \cdot \log I_0/I = 1/k \cdot D \quad (3)$$

where *k* is a constant for a given solute in a cell of given thickness. As has been shown, Beer's law may be effectively true for heterogeneous light, such as might be transmitted by a filter of appropriate characteristics, if the variation of absorption coefficient over the wave-length range incident on the sample is not too great. In such a case, a preliminary calibration set of measurements at different concentrations will allow the calculation of *k*; and, in any subsequent determination, the concentration of an unknown can be determined in the same apparatus from the measured density by multiplying by the factor 1/*k*. Standards then need no longer be made, after the preliminary calibration, so long as no change is made in the apparatus, such as the replacement of lamp, filter, absorption cell,

or photocell. If equation (3) is known to hold for the system, any recalibration would require only a single determination.

The experimental test of equation (3) is the linearity of the plot of density against concentration. In practice, a linear relation is found for many systems over a more or less great range of concentrations, the slope being k . For example, Ashley,⁴ using a Pulfrich photometer with a filter transmitting the mercury green line $546\text{ m}\mu$, found permanganate, as produced by the periodate method of Willard and Greathouse, to show a density linear with concentration over the range 0.01 to 2% (see Fig. 2). It frequently happens that with light filtered from a white source, linearity is obtained at lower concentrations, with deviations at higher concentrations.

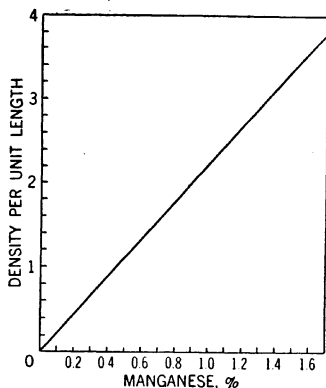


Fig. 2.—Calibration curve for iodate method for manganese using a filter transmitting mercury green line $546\text{ m}\mu$ (after Ashley⁴).

Figure 3 shows typical density-concentration calibration curves for the photometric analysis of dilute solutions of iodine in various solvents. The iodine was the product of a photochemical reaction⁵ and the photometric arrangement was so disposed as to permit measurements in the reacting system *in situ*. The ease with which photoelectric photometry permits analysis without requiring removal of the material from the system is one of the advantages of this method in research over the older colorimetric methods and also over most subjective photometric methods. The filter used in the measurements of figure 3 was a combination of saturated copper sulfate,

5% solution of aqueous sodium chromate, and an aqueous solution of malachite green of concentration adjusted to transmit the region of maximum absorption of iodine, contained in separate cells 8 mm. thick. It would have been quite impossible to measure the iodine in the very pale pink solutions by subjective comparative colorimetry, with adequate accuracy.

An otherwise suitable colorimetric system need not be dismissed from consideration for absolute colorimetry if it shows deviations from Beer's law. If the prime requirements of speedy development of the color, reproducibility, and lack of interference by other materials likely to occur in the analysis are met, a nonlinear calibration curve can be tolerated.

⁴ S. E. Q. Ashley, *Ind. Eng. Chem., Anal. Ed.*, **11**, 72 (1939).

⁵ W. West and J. Fitelson, *J. Am. Chem. Soc.*, **62**, 3021 (1940).

One should make sure, however, that nonlinearity in such a case is really intrinsic, and not merely due to a poor choice of filter or to some other correctable factor. Linearity of density with concentration is good evidence of an essentially simple colorimetric system and properly adjusted apparatus.

Another advantage of the photometric method over the older methods of color comparison is the very material gain in sensitivity that can be realized. Especially is this true with objective photometry for the colors like pale yellows and pinks whose discrimination offers great difficulties to the eye. In photometry, attention is directed toward light in the absorption bands

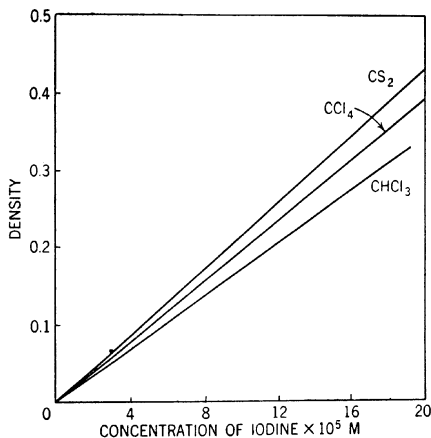


Fig. 3.—Typical density-concentration curves.

rather than in the regions of high transmission. The method depends on the difference in the amount of light entering the receiver (the eye, photoelectric cell, etc.) when the absorbing sample and the nonabsorbing solvent are placed, respectively, in the beam. This difference is obviously greatest for the wave lengths most intensely absorbed, that is, at the maximum of the absorption band; and the presence of light in the measured beam which is not relatively highly absorbed merely reduces sensitivity for small concentration differences. Photometry is therefore carried out with the use of filters transmitting only the radiations most absorbed, or even, in special cases, by means of spectrophotometers of the type described in Chapter XXI.

The formal proof of the above statement for systems adhering to Beer's law is to form the derivative of the density, D , as a function of the concentration:

$$D = \epsilon cd$$

$$\partial D / \partial c = \epsilon d$$

so that, at constant cell thickness, the change in density effected by a given change in concentration is the greater the greater the molar extinction coefficient, and, for a given substance at a given wave length, the absolute change in density with concentration is the greater the greater the thickness of the absorbing layer.

4. Dichromatism

Some colored substances appear differently colored in transmitted light according to the thickness of the layer. Thus, a thin layer of the dye pinakryptol green in aqueous or alcoholic solution appears green, a thicker layer reddish. The phenomenon is called dichromatism, and it is obvious that such solutions are not amenable to colorimetric determination by the balancing method in white light. No difficulty is afforded in the photometric method, provided a filter is used which absorbs one of the bands. In the balancing method, too, the difficulty could be eliminated by interposing a filter, say between the ocular of the comparator and the eye, to absorb the light of one of the transmission bands.

The explanation of dichromatism lies in the existence of two transmission bands of different intensities and in spectral regions to which the eye is unequally sensitive. Pinakryptol green absorbs strongly in the blue, and has a weaker band in the orange and near-red. It therefore transmits green and deep red, the fraction of green light passed by a given thickness being smaller than that of red. In thin layers, the smaller amount of green than red transmitted is more than balanced by the greater sensitivity of the eye to green, with the result that the color transmitted appears greenish; thick layers absorb practically all the green but transmit enough deep red to give a red color. The explanation of the phenomenon shows that it may be observed if two colored substances are present with absorption bands in different spectral regions.

5. Deviations from Beer's Law

It has already been mentioned that apparent deviations from Beer's law, in the sense that the density increases less rapidly than linearly with the concentration, will be met with when the substance has a variable absorption coefficient over the wave-length region used in the measurements of transmission. In the strict sense, one need not expect the law to be true for any system at sufficiently high concentrations, owing to solute-solute interactions, but often such deviations are small. Much more pronounced deviations arise when the colored substance is one of the participants in a dynamic equilibrium, and the degree of conversion to the colored product

depends on the concentration. Equilibria involving two substances in a constant ratio of concentrations do not cause deviations. Association, dissociation, and complex formation can make the law invalid. Extreme deviations occur in aqueous solutions of many dyes, where not only may associative processes produce deviations within the absorption band, but new bands may appear with increasing concentration at the expense of those occurring in dilute solution. The effects of stray light in causing apparent deviations from both Beer's and Lambert's laws are discussed in Chapter XXI, page 1327.

While adherence to Beer's law over a useful concentration range is a desirable feature in a colorimetric reaction, it has already been pointed out that it is not an absolute essential in a system otherwise acceptable in reproducibility, freedom from fading, and lack of susceptibility to change in the presence of adventitious substances. A nonlinear calibration curve of density against concentration can be relied upon as well as a linear one, so long as the apparatus remains unchanged. A recalibration following any change in the apparatus, however, will require the determination of several points. Even if the density-concentration curve is substantially linear as obtained with a filter photometer, its slope will not in general be a characteristic of the chemical system, but will depend on the light source, filter, and receiver used. Only with complete standardization in these respects would it be thinkable to use a curve obtained with other apparatus as the basis of a determination; and in any circumstances proof of standardization would demand verification of at least a few points on the curve.

In comparative colorimetry by the balance method, the validity of equation (3) depends on Beer's law being followed. In the development of a specific method using the balancing procedure, the truth of the equation should be verified over a range of concentration by finding the points of match of various known concentrations against a fixed concentration and thickness. If the equation is not valid, an empirical calibration curve must be used.

6. Photometric Accuracy in Colorimetry

The over-all accuracy of a colorimetric method depends: (a) on the nature of the chemical reactions employed in developing the color, their degree of completeness, the susceptibility of the color to fading etc.; and (b) on the accuracy of the strictly photometric measurement or of the color comparison. Errors of the first class are unique for any specific analytical method, and must be found in the development of the method; but some general statements can be made on the influence of the errors in the purely colorimetric determination on the accuracy of the determination of the concentration (the analytical accuracy). A detailed analysis and experi-

mental test of the photometric errors in objective colorimetry have been made by Ringbom^{6, 7} and coworkers. A careful experimental investigation of the analytical errors as a function of the transmitted light in the use of the Pulfrich visual photometer (page 1429) is reported by Ashley.⁴ Reference should be made to these articles for further details than those furnished in the following discussion.

A. SUBJECTIVE METHODS

In subjective methods, whether comparative, as in the balancing method (Duboscq), or absolute, as in the Pulfrich, the sensitivity of the eye to differences of brightness in fields of the same color is the limiting factor controlling the accuracy. Under very good conditions, the trained eye can detect a brightness difference of about 1% in two juxtaposed fields simultaneously observed, irrespective of the absolute brightness over a large range (the Weber-Fechner law). For white light, the range of brightness for which the eye has approximately constant relative discrimination is about one to 250 foot-lamberts, where the foot-lambert is the brightness of a perfectly reflecting, perfectly diffuse surface illuminated by a point source of one standard candle at a distance of one foot from every point in the surface. The eye is differently sensitive for different colors: the sensitivity is a maximum, at daylight levels of illumination, at about wave length 553 m μ , in the green, and falls off rapidly to longer and shorter wave lengths (p. 1268).

If the colored material conforms to Beer's law, the general equation for the effect of a small error in the transmitted intensity can be found by differentiating equation (3), which gives for the relative error in the concentration, in the case where the effect of fluctuations in the incident intensity is eliminated:

$$\frac{dc}{c} = - \frac{1}{\ln I_0/I} \frac{dI}{I} = - \frac{0.434}{D} \frac{dI}{I} \quad (4)$$

In subjective methods, the relative error in the transmitted intensity is constant over a certain range of intensities, so that the error in the concentration is inversely proportional to the density. In practice, the loss in brightness discrimination at densities above about 1 (10% transmission), prevents the use of very high densities, and accuracy is greatest for transmissions of 10 to 20% (D 1 to 0.7). Assuming very favorable conditions (an unfatigued trained eye, no distracting lights in the room, juxtaposed photometric fields in the color range of maximum sensitivity, adequately large and with a sufficiently long junction line), the percentage error in I

⁶ A. Ringbom, *Z. anal. Chem.*, **115**, 323 (1938).

⁷ A. Ringbom and F. Sundman, *Z. anal. Chem.*, **115**, 402 (1938).

will be about 1%, making an analytical error of about 0.5%. In general, careful work with a good subjective colorimeter may be expected to have an analytical error of about $\pm 1\%$.

B. OBJECTIVE METHODS

In objective measurements, for instance, with photoelectric cells or thermopiles, the limit of discrimination between two transmissions is determined by the smallest absolute difference in energy that can be detected by the instrument. By increasing the intensity of the source and the sensitivity of the galvanometer, the limit of discrimination can be made, within certain limits, arbitrarily small. Inconstancy of the sensitivity of the cell with time, temperature, and the amount of preillumination, inconstancy in the light source, as well as the nature of the electric circuit, become limiting factors in determining the analytical error.

In equation (4), dc/c is no longer independent of the transmitted intensity, and its variation with I can be expressed by:

$$\frac{dc/c}{dI} = \frac{0.434}{DI} \quad (5)$$

To find the percentage transmission associated with minimum relative error in the concentration produced by a given error in the transmission, the second derivative:

$$\frac{d \cdot dc/c}{dI^2} = \frac{d^2 \ln c}{dI^2}$$

is equated to zero. The solution is:

$$\ln I_0/I = 1, \text{ or } \log_{10} I_0/I = D = 0.434 \quad (6)$$

In an objective photometric measurement in a system conforming to Beer's law, then, a given error in the transmission causes least error in the concentration at a density of 0.434, or 36.8% transmission, a result found by Ringbom.^{6,7}

Equation (5) shows that, at 36.8% transmission, an error of 1% in transmission causes a relative error of $\pm 2.72\%$ in concentration. The minimum in the analytical error as a function of transmission is flat, so that 30-60% absorption can be regarded as in the optimum region. An error of $\pm 0.5\%$ in the concentration requires an error in the transmission, at about the optimum value, no greater than about 0.2%. This can be effected with well-designed photometers using photovoltaic cells. With vacuum emission cells and a vacuum-tube voltmeter, a photometric precision of 0.03 to 0.05% is reported.⁸

⁸ G. P. Rowland, Jr., *Ind. Eng. Chem., Anal. Ed.*, **11**, 442 (1939).

7. Optimum Concentration, Sensitivity, and Range in Objective Photometric Colorimetry

The condition that a given error in the measurement causes least error in the estimated concentration at transmissions of about 38%, if Beer's law is adhered to, with a knowledge of the molar absorption coefficient, allows the determination of the optimum concentration. From equation (1):

$$(cd)_{\text{optimum}} = D_{\text{optimum}}/\epsilon = 0.434/\epsilon \quad (7)$$

The second column of table I contains the values of the molar concentrations which yield densities of 0.434 at thicknesses of 1 cm. For other

TABLE I
OPTIMUM AND DETECTABLE CONCENTRATIONS

ϵ	c_{optimum} at 1 cm., molar	$c_{\text{detectable}}$ at 1 cm., molar
1	4.34×10^{-1}	10^{-3}
10	4.34×10^{-2}	10^{-4}
10^2	4.34×10^{-3}	10^{-5}
10^3	4.34×10^{-4}	10^{-6}
10^4	4.34×10^{-5}	10^{-7}
10^5	4.34×10^{-6}	10^{-8}

lengths of absorbing layer, the optimum concentrations are inversely proportional to the thicknesses.

The lower limit of detectability of a substance, insofar as it depends on the photometric measurements, is determined by the smallest concentration that will give an absorption detectably different from that of a blank. The third column of table I contains the concentrations in a cell 1 cm. long that will produce an absorption of 0.2%, which is within the capacity of a good photoelectric colorimeter. Increase in the length of the cell in the case of feeble absorption causes a nearly proportional increase in the sensitivity. It is seen that highly absorbing substances ($\epsilon = 10^4$ or 10^5) can be detected at concentrations of 0.05 to 0.005 γ per ml. or less.

The concentration range over which measurements can be conveniently made varies with the system. If a linear density-concentration relation exists (at constant cell thickness) over a restricted range of reasonable spread, the convenience of the straight-line calibration suggests working in that range. The range of linearity may be very great, over a 200-fold concentration variation, or more. It would then be appropriate to use different cell lengths for different concentrations, and to plot as the calibration curve the density per unit cell length against the concentration.

III. PHOTOELECTRIC PHOTOMETRY

The general principles of photoelectric photometry are discussed in Chapter XXI on page 1314, which includes a detailed account of the use of electron emissive tubes. In filter photometry for analytical purposes, it is rarely necessary to use very low intensities and the self-generative photovoltaic cell can often be used conveniently. Most commercial photoelectric colorimeters employ photovoltaic cells. In improvising a photometer for ordinary analytical purposes, this cell is simplest and least expensive. On the other hand, where sensitivity at very low intensities is required, as in some measurements of fluorescence, the amplified phototube is indicated. In very precise work, also, the vacuum type of phototube seems to have advantages over the photovoltaic cell.⁹

1. Characteristics of Photovoltaic Cells

The photovoltaic cell consists of a thin deposit of a semiconductor, as selenium or copper oxide, on a metal base, *e. g.*, iron or copper. The semiconductor is usually covered with a very thin transparent metallic coating. The whole is contained in a moistureproof case, with a glass or other window, or with the sensitive surface covered with a protective lacquer coating. The terminals of the cell are connected to the metal electrodes on the two sides of the semiconducting layer.

Exposure of the active element to light of the appropriate frequency causes a flow of electrons to take place from the semiconductor to one of the metal electrodes in contact with it. The term "barrier layer" refers to the rectifying properties of these elements in the dark, electrons passing freely from the metal to the semiconductor, but not in the opposite direction. When the cell is illuminated, it may be supposed that electrons are freed in the semiconducting layer, probably in numbers proportional to the intensity of the exciting light. As part of a circuit, however, the cell acts both as a source of e. m. f. and as a resistance shunting the external circuit. In the Photronic Cell, the value of this shunting resistance decreases nonlinearly with intensity of illumination from about 7000 ohms in the dark to about half that at about 100 foot-candles intensity of illumination (p. 1267), diminishing further at higher intensities, with the result that the current is proportional to the illumination only at low values of the external resistance (see Fig. 4). In an external circuit of sufficiently low resistance, such as a low-resistance microammeter, the response is seen to be practically linear with intensity up to moderate intensities, and of magnitude about 120 μ a. per lumen received by the cell from a tungsten lamp at about 3000° K.

At moderate intensities of illumination (above about 10 foot-candles in

⁹ R. H. Müller, *Ind. Eng. Chem., Anal. Ed.*, **11**, 1 (1939).

selenide cells, like the Photronic), the *open-circuit electromotive force* is proportional to the logarithm of the intensity. Since the concentrations of absorbing solutions obeying Beer's law are proportional to the logarithm of the reciprocal of the transmission, the possibility arises of using the logarithmic relation between open-circuit e. m. f. and intensity for the di-

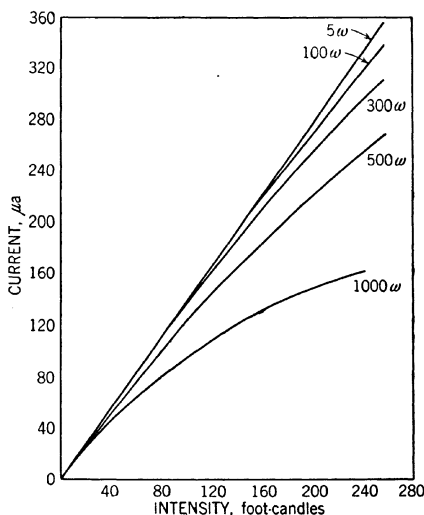


Fig. 4.—Current-intensity relation for Photronic Cell for various external resistances.

rect readings of concentrations on a linear scale. Such a circuit is described by Müller and Kinney¹⁰ (see Fig. 9, page 1417).

Figure 5 shows the *response* of a Photronic Cell to equal incident energies of different wave lengths. The maximum sensitivity is at about 580 $m\mu$, falling off rapidly toward the red to a small value at 700 $m\mu$ and less rapidly toward the shorter wave lengths. With suitable windows, selenium photo-voltaic cells have a useful sensitivity in the ultraviolet. For ultraviolet measurements, the device of converting the short-wave radiation to visible fluorescent light of color near the maximum of sensitivity may also be used (see Chapter XXI, page 1317).

The so-called cuprous-oxide back-wall cell (*i. e.*, a cell in which the rectifying barrier layer is adjacent to the metal base on the side of the semi-

¹⁰ R. H. Müller and G. F. Kinney, *J. Optical Soc. Am.*, 25, 342 (1935).

conducting layer away from the incident light, in distinction to the "front-wall" cell, in which the rectifying layer is adjacent to the front electrode) has a high, but rather temperature-dependent sensitivity in the near-infrared, with a maximum at about $800\text{ m}\mu$ and appreciable sensitivity at $1000\text{ m}\mu$.

Photovoltaic cells show *fatigue*, that is, a diminution in the photocurrent with continuous exposure to the exciting light. The phenomenon is com-

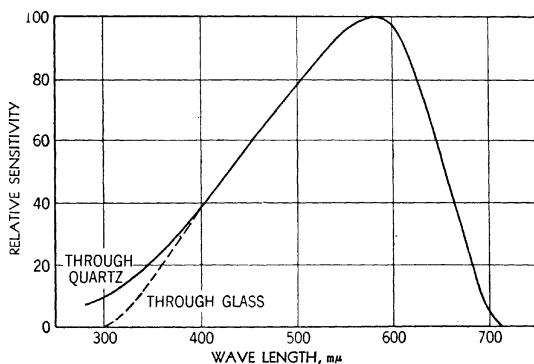


Fig. 5.—Spectral sensitivity of a Photronic Cell.

plex, and depends on the nature of the surfaces, the intensity of illumination, the external resistance, and the resistance of the cell. Experiments are recorded, for example by Lange,¹¹ which show a 30% decrease in sensitivity of a selenium photovoltaic cell after ten minutes exposure at 10,000 foot-candles (direct sunlight) and a 2% decrease at 3 foot-candles. Ultimate recovery of the original sensitivity after keeping in the dark appears to occur.

The existence of fatigue makes it advisable to keep a cell intended for photometric purposes from exposure to bright light, to use as low working intensities as convenient, and not to make measurements of a weak source immediately after exposure of the cell to a much stronger one.

Photovoltaic cells have an appreciable temperature coefficient of sensitivity, and for highest precision should be maintained at constant temperature.

¹¹ B. Lange, *Photoelements and Their Application*. Reinhold, New York, 1938, p. 164.

2. Circuits for Photovoltaic Photometers

A. SIMPLE PHOTOMETER

Figure 6 shows the essentials of a simple photometer which is easily improvised. The unknown solution and the pure solvent are placed in matched cells mounted side by side on a carriage which can be reproducibly moved by sliding between rails, etc., so that they can be placed alternately in the light beam. Suitably placed stops set the positions.

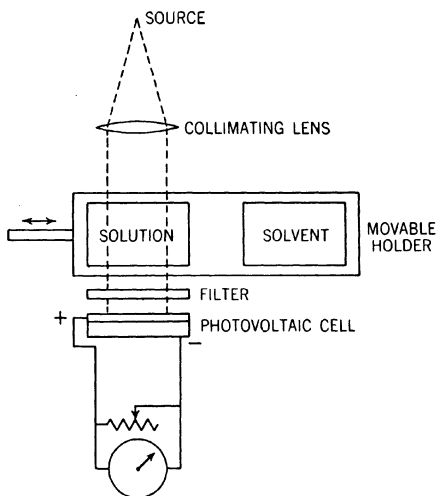


Fig. 6.—Simple photoelectric photometer.

For most purposes tungsten lamps are convenient sources of light. Automobile lamps are often suitable, operated from batteries or from the a. c. mains by means of a step-down transformer. Constant-voltage transformers materially improve the constancy of the source. If batteries are employed for a photometer in constant use, it is convenient to trickle-charge them from the d. c. line.

The mercury arc, in the form of the General Electric H4 lamp or a similar lamp, is an intense and steady source useful in some applications. Its spectrum has strong lines at 577, 579 (yellow), 546 (green), 436 (blue), and 405 m μ (violet), besides 366 m μ (ultraviolet). With suitable filters, prac-

tically monochromatic light can be obtained from this source. Since this lamp becomes very hot in operation, it should be well insulated thermally from the rest of the photometer or mounted apart from the portion containing the solutions and photocell.

Mercury lamps of the H type increase rapidly in intensity for about fifteen minutes after starting, and to a small degree later.^{11a} In circuits not compensated for changes in light intensity, sufficient time should be allowed after starting the lamp to insure substantial constancy.

A simple lens or spherical mirror of two or three inches focal length renders parallel the light from the source. The beam should be diaphragmed so as not to strike the side of the cell.

Cells with plane fused windows, as supplied commercially by several manufacturers, are preferable to those with cemented windows.

The photocell can be mounted in a radio-tube socket. The meter should have a resistance of 100 ohms or so: the sensitivity required depends on the intensity of the source, the amount of light transmitted by the filter, and the wave length at which measurements are to be made. Usually an instrument giving full-scale deflection for a few microamperes will be adequate. The meter can be shunted with coarse- and fine-adjusting rheostats to arrange that full-scale deflection is secured at 100% transmission. The readings may overshoot; the final steady value after a few seconds is to be accepted.

In this apparatus, if the source is constant, the photocurrent proportional to the intensity of illumination, and no error present due to fatigue, the ratio of the meter deflections when solvent and solution are placed, respectively, in the beam is the quantity whose logarithm is the density.

In methods which depend on the proportionality of the photocurrent to intensity, this relation should be checked at the intensities to be used in the photometer. The obtaining of a straight line for the density-concentration relation, as measured in the instrument, for a substance, *e. g.*, potassium permanganate, known to obey Beer's law^{12,13} may be regarded as satisfactory proof of linearity in the intensity-current relation. Or the relation can be tested directly, by using, for instance, the inverse square relation between intensity and distance from a point source. The minimum distance from source to cell in this test must be large enough for the relation to be substantially true. A variable aperture over a lens, calibrated screens placed in the optical path, or polarizing apparatus are other devices permitting intensity to be varied in a known ratio.

^{11a} G. Wald, *J. Optical Soc. Am.*, **35**, 189 (1945).

¹² S. E. Q. Ashley, *Ind. Eng. Chem., Anal. Ed.*, **11**, 72 (1939).

¹³ R. H. Müller, *Ind. Eng. Chem., Anal. Ed.*, **7**, 361 (1935). J. P. Mehlig, *ibid.*, **7**, 361 (1935).

B. POTENTIOMETRIC METHOD

In figure 7 a circuit is shown which, by a potentiometric measurement of the voltage drop produced by the photocurrent in an external resistance of about 100 ohms, permits readings to be made on a graduated slide wire. With the solvent in the beam, the slide wire is set at 100, and the working current of the potentiometer adjusted for zero reading of the galvanometer, one of high voltage sensitivity.

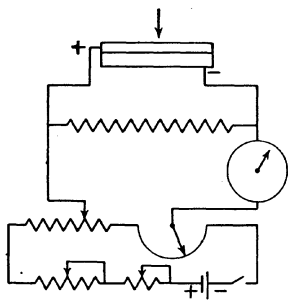


Fig. 7.—Potentiometric arrangement for single-cell circuit.

C. ZERO-POTENTIAL CIRCUIT

Wood¹⁴ has emphasized the increase in stability and in linearity of response with intensity and the decrease in the temperature coefficient of sensitivity which are gained when a photovoltaic cell is made to function without a potential drop across the cell. In figure 8, the potential drop across the resistance *AB* can be adjusted equal to that across the microammeter; the poles of the photocell are then at the same potential. Shorting the cell by closing switch *S*₁ will then have no effect on the galvanometer reading. Cell *C* may be a dry cell; the potentiometer, a radio potentiometer of 10,000 ohms, with a fine adjustment of 1000 ohms, the microammeter 100 or 150 ohms or so. The absorption cell with sol-

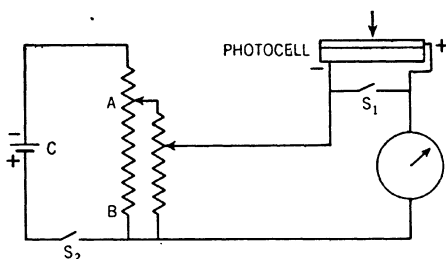


Fig. 8.—Zero-potential circuit.

vent is placed in the light path, switches *S*₁ and *S*₂ are closed, and the current from *C* is adjusted so that full-scale deflection is indicated on the

¹⁴L. A. Wood, *Rev. Sci. Instruments*, 7, 157 (1936).

meter. S_1 is opened, and the intensity of the light adjusted by means of an iris diaphragm at the lens or by other means until the meter reads full deflection. The reading, I_0 , for no absorption is thus made under the condition of zero potential. On introducing the sample into the beam, the new condition of balance is found by reducing the current from the dry cell until, on opening and closing S_1 , no change in deflection occurs. Given a steady source, the transmission of the sample is the ratio of the second to the first meter reading.

D. OPEN-CIRCUIT E. M. F. MEASUREMENT

Figure 9 shows the circuit of Müller and Kinney,¹⁰ analogous to the well-known arrangement of Hildebrand used in electro-metric titrations. At intensities of light higher than required to produce a photo e. m. f. greater than about 60 mv., the e. m. f. is proportional to the logarithm of the intensity. In figure 9, the millivoltmeter is so placed that its readings increase with the concentration of the absorbing substance, linearly, if the system obeys Beer's law.

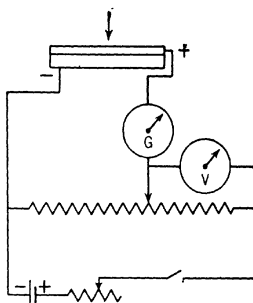


Fig. 9.—Measurement of open-circuit e. m. f. of photovoltaic cell.

E. TWO-CELL CIRCUITS

Differential and null circuits are often used in photoelectric photometry for the purpose of gaining sensitivity or stability. In differential circuits,

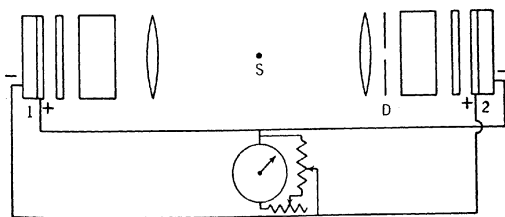


Fig. 10.—Two-cell photometer.

the difference between two oppositely directed photocurrents, for example, those yielded by a "standard" photocell and a "measuring" cell, is directly read by means of a meter; in null circuits, the meter is used simply to indicate equality of the two opposing currents, the equalization being performed

by some device provided with a reading scale, as a variable aperture, neutral wedge, variable depth of liquid, or a variable resistance or potentiometer.

One of the simplest two-cell circuits (for photovoltaic cells), shown in figure 10, is described by Lange.¹⁵ Cells 1 and 2 are illuminated by a parallel beam from source *S*. With solvent in front of both cells, the photocurrents are adjusted so that the galvanometer reads zero, by turning the lamp, by adjusting the position of a sliding slot over the lamp, or by using the variable diaphragm, *D*. Cell 2 is then covered with an opaque screen, and the shunts across the meter adjusted until the reading is 100, corresponding to 100% absorption of the material in front of cell 2. If now the sample is introduced in front of cell 2, the meter reading gives the percentage absorption directly, provided the photocurrents are linear over the range of intensities used. It will be seen that, although the zero reading in

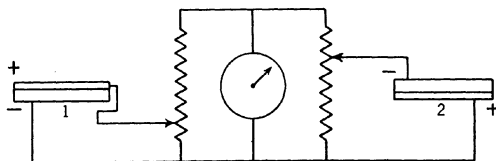


Fig. 11.—Brice's compensated circuit.

this method is independent of the fluctuations of the source, the deflections are not, so that a constant source is required. The method, however, can be made a null one by compensating for the absorption of the unknown, placed in front of cell 1 by means of variable diaphragm *D*, graduated in transmission or other suitable units. The arrangement then becomes of the zero-potential type and independent of fluctuations of the source.

If the absorption to be measured is small, the direct-deflection method can be made more sensitive by first adjusting the apparatus as described, so that a deflection of 1 on the scale corresponds to 1% absorption, then reducing variable diaphragm *D* until only 10%, say, of the light reaches cell 2, and then increasing the shunt resistance until a reading of 100 is attained. One scale division then corresponds to 0.1% absorption.

Figure 11 shows a modification due to Brice,¹⁶ who made a careful study of compensated circuits. With Photronic Cells, and an intensity of illumination yielding photocurrents of 20 to 50 μ a., potentiometer resistances of about 50 ohms were found suitable, with a galvanometer of 22 ohms resistance. This circuit is also, at balance, independent of fluctuation of the

¹⁵ B. Lange, *Physik. Z.*, **31**, 964 (1930).

light intensity.¹⁷ With solvent in front of measuring cell 2, that is, with 100% transmission, the measuring potentiometer is set at 100, and the compensating potentiometer attached to cell 1 is adjusted for zero deflection in the galvanometer. On introducing the sample in front of cell 2, the position of balance of the measuring potentiometer gives directly the transmission of the unknown.

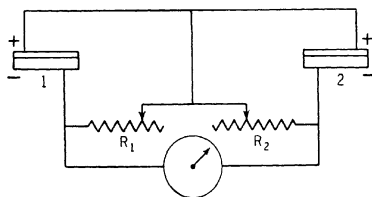


Fig. 12.—Wilcox's balanced circuit.

As a final example of a frequently used balanced circuit, that of Wilcox¹⁸ is shown in figure 12. At balance, the potential-drops across their respective resistances produced by the two photocurrents are equal and opposite.

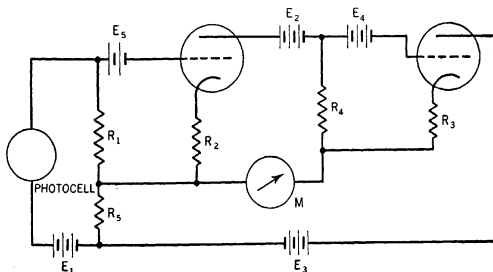


Fig. 13.—Amplification of photovoltaic cell after Rittner.¹⁹

The slide-wire of R_2 is set so that its scale reads 100 when the solvent is placed in front of the measuring cell 2, and balance is secured by adjusting R_1 . The position of rebalance in R_2 on introduction of the sample gives the transmission directly. This is not a "zero-potential" circuit, and it is particularly important to keep resistances R_1 and R_2 small. At balance, fluctuations of light intensity are automatically compensated.

¹⁸ B. A. Brice, *Rev. Sci. Instruments*, **8**, 279 (1937).

¹⁷ R. H. Müller, *Ind. Eng. Chem., Anal. Ed.*, **11**, 1 (1939).

¹⁹ L. V. Wilcox, *Ind. Eng. Chem., Anal. Ed.*, **6**, 167 (1934).

F. AMPLIFICATION OF PHOTOVOLTAIC AND PHOTOCONDUCTIVE CELLS

Rittner has described electronic circuits which circumvent the decrease in current sensitivity of photovoltaic and photoresistive cells with increasing load resistance by automatically maintaining nearly constant voltage across the cell, and also act as amplifiers.¹⁹ One of these circuits is shown diagrammatically in figure 13. The photocurrent causes a potential fall across the resistance R_1 which is fed into a two-stage amplifier. A por-

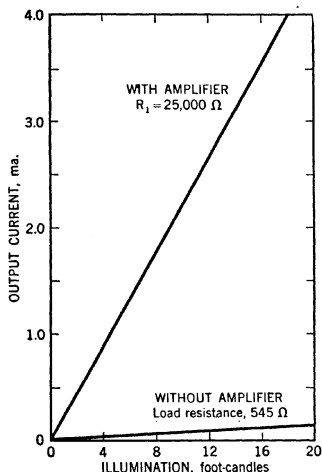


Fig. 14.—Amplification of a Photronic Cell.¹⁹

tion of the output of the second stage is fed back into the photocell circuit by means of the resistance R_5 to maintain nearly constant voltage across the cell.

$R_1 = 25,000$ ohms; $R_2 = R_3 \doteq 500$ ohms; $R_4 = R_5 \doteq 800$ ohms; $E_2 = E_3$ are plate-supply batteries of 90 volts; E_4 is a variable-bias voltage which can be adjusted to balance the circuit so that the 0-5 millimeter, M , registers zero when no signal is applied; E_5 is a variable voltage supply used to balance out dark currents if present; E_1 is a variable voltage supply to maintain any desired voltage across the photocell. In the initial adjustment of the circuit $R_4 = R_5$ are set so that the change in voltage across R_4 produced by illumination of the cell is equal, as nearly as possible, to the voltage change across R_1 . A twin triode, such as a 6F8-G, can be used.

¹⁹ E. S. Rittner, *Rev. Sci. Instruments*, 18, 36 (1947).

This circuit was found to be stable so long as R_1 did not exceed 25,000 ohms. In figure 14 are shown the current output of a Photronic Cell without amplification and the output of the amplifier connected to the photo-cell.

The photometric methods used with phototubes are essentially the same as those described for use with photovoltaic cells. For further details on well-tested and reliable circuits for photoemissive tubes, reference is made to the article of Müller.²⁰

3. Filters

As has been mentioned before, the sensitivity of photometric methods of analysis is increased by filters transmitting only the light absorbed by the analysand; moreover, only with the use of filters can one expect a linear density-concentration relation. The permissible wave-length breadth of the transmission band of the filter is the greater the more slowly the extinction coefficient of the substance varies with wave length.

In many cases, the absorption bands of dissolved substances are broad and it is possible to obtain a substantially linear concentration-density curve with ordinary color filters with transmission maxima near the absorption maxima of the substances. Figure 15, from data published by Müller and Burtzell,²¹ shows the transmission of a set of Corning Glass Filters from which a suitable choice can be made for most purposes. Thus, the obvious choice for the chromium complex of figure 1, curve *c* is the *G* filter, while for the cuprammonium complex *E* or *F* is indicated. The dye (curve *B*), because of the relative narrowness of its band near 600 $m\mu$, would be more difficult to fit, and no combination of the filters in figure 15 would be very suitable.

The use of a line spectral source like the mercury arc with appropriate filters permits a closer approximation to monochromatic light than can be secured from filtered white light. For example, filter *G* of figure 15, combined with a neodymium glass filter to remove the yellow lines from the mercury arc, would furnish a close approximation to monochromatic radiation of wave length 546 $m\mu$. "Mercury monochromatic filters" are manufactured by the principal suppliers of color filters.

Glass filters for the visible, near-infrared and ultraviolet are made by *Corning Glass Works*, Corning, N. Y., by *Schott*, Jena, and by *Chance Bros.*, England. The Schott filters are normally procurable from *Fish-Schurman Corp.*, New York. Dyed gelatin filters are supplied by *Eastman Kodak Co.*, Rochester, N. Y., under the name of Wratten Filters and by *Ilford*,

²⁰ R. H. Müller, *Ind. Eng. Chem., Anal. Ed.*, 11, 1 (1939).

²¹ R. H. Müller and A. T. Burtzell, *Mikrochemie*, 28, 210 (1940).

Ltd., London. Booklets are published by the various manufacturers containing information on the transmission-wave-length characteristics of their products. Gelatin filters usually have sharper cutoffs than glass, but lack the resistance to heat, water, etc., of the latter. Glass filters may also be more stable, although some are attacked by atmospheric moisture. In use, gelatin filters should be kept away from hot light sources; for instance, they may be placed immediately in front of the photocell or other receiver.

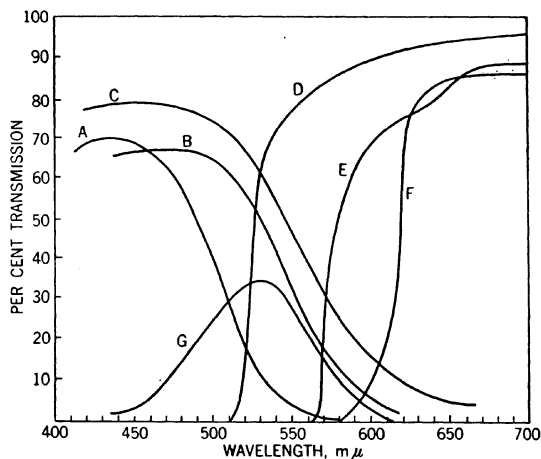


Fig. 15.—Transmission of some Corning filters. *A*, dark shade theatre blue, 3.44 mm.; *B*, signal green, 6.04 mm.; *C*, light shade blue-green No. 428, 4.0 mm.; *D*, heat-resisting yellow shade yellow, No. 351, 2.93 mm.; *E*, heat-resisting red shade yellow; *F*, red, No. 245; *G*, sextant green. (After Müller and Burtzell.^{21a})

The possible transmission in the near-infrared of filters intended for the visible should be remembered if the receiver is sensitive to infrared radiation. Infrared light can be partially removed by the Corning Aklo filters, or by a layer of molar ferrous ammonium sulfate about two centimeters thick.

Colored solutions can be used as filters in plane-parallel glass cells. Table II lists some liquid filters which can be used to isolate mercury lines. The isolation of the ultraviolet lines of mercury is also given, since photometric filter analysis can be extended to that region; these filters may also be useful in fluorescence analysis. A number of transmission filters for ultraviolet radiation are described by Kasha.^{21a}

^{21a} M. Kasha, *J. Optical Soc. Am.*, **38**, 929 (1948).

TABLE II
 LIQUID FILTERS FOR MERCURY LINES

Wave length, $m\mu$	Region	Filter
577-579	Yellow	Dilute chrysoidine + eosine added until green disappears ^a $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$ 11.1 g. per 100 ml. water, 1 cm., + $\text{K}_2\text{Cr}_2\text{O}_7$ satd. soln., 2.5 cm. (transmits 36% of 577 $m\mu$ and some 546 $m\mu$) ^b
546	Green	$\text{K}_2\text{Cr}_2\text{O}_7$ + neodymium ammonium nitrate, in separate vessels or + Corning neodymium glass filter ^a
436	Blue	Dilute iodine in CCl_4 + NaNO_3 in water ^a KMnO_4 , 0.84 g. per l. water, 1 cm., + $\text{CuCl}_2 \cdot 2 \text{H}_2\text{O}$ 37.5 g. per 100 ml. water, 0.5 cm. (transmits 12% 436 $m\mu$) ^b
405, 408	Violet	$\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$, 0.44 g. per 100 ml. 2.7 M NH_4OH , 10 cm. + I_2 , 0.75 g. per 100 ml. CCl_4 , 1 cm. + quinine hydrochloride, 1 g. per 100 water, 2 cm. ^b (frequent renewal of iodine and quinine necessary) ^b
366	Ultraviolet	$\text{Cu}(\text{NO}_3)_2 \cdot 6 \text{H}_2\text{O}$, 16.7 g. per 100 ml. water, 0.5 cm., + Wratten No. 18 (transmits 10% of 366 $m\mu$, very little 335 or 405 $m\mu$) ^b (The most convenient filters for general near-ultraviolet are the ultraviolet transmitting glasses of the <i>Corning Glass Works</i> and <i>Chance Bros.</i> , England, containing nickel oxide.)
313	Ultraviolet	$\text{NiSO}_4 \cdot 6 \text{H}_2\text{O}$, 46 g., + $\text{CoSO}_4 \cdot 7 \text{H}_2\text{O}$, 14 g. per 100 ml. water, 3 cm., + potassium hydrogen phthalate, 5 g. per l. water, 1 cm., in quartz or clear Corex (<i>Corning</i>). (Transmits 80% at 313 $m\mu$, 3% at 334. The salts must be very pure, especially free from iron. Renew phthalate frequently.) ^b NiCl_2 , 0.178 M , 5 cm., + K_2CrO_4 , 0.0005 M , 5 cm., + 0.0245 M potassium hydrogen phthalate, 1 cm., 0.0005 M red purple Corex, 5 mm. (<i>Corning</i> No. 9863). (Transmits 25% at 313 $m\mu$, reducing extraneous ultraviolet to less than 0.01%.) ^c
254	Ultraviolet	I_2 , 0.108 g., + KI , 0.155 g. per l. water, 1 cm., + NiSO_4 and CoSO_4 as for 313 $m\mu$ (transmits about 20% at 254) ^b A quartz cell containing chlorine at about 6 atm. (obtainable from the <i>Hanovia Chemical & Mfg. Co.</i> , Newark, N. J.) transmits 254 $m\mu$ freely and absorbs most of the longer ultraviolet; with bromine in a separate vessel, much of the visible can be eliminated. The transmission at 254 $m\mu$ is diminished if the two gases are placed in the same vessel.

^a R. W. Wood, *Physical Optics*. Macmillan, New York, 1934, p. 15.^b W. A. Noyes and P. A. Leighton, *The Photochemistry of Gases*. Reinhold, New York, 1942, p. 69.^c R. E. Hunt and W. Davis, Jr., *J. Am. Chem. Soc.*, **69**, 1415 (1947).

Recently filters have been developed which depend on *interference* for their selectivity. *Transmission interference filters*, transmitting narrow bands of "half-width*" 100 to 150 Å. consist of two thin, semitransparent, parallel metallic films, usually silver for the visible spectrum, separated by a layer of dielectric, whose thickness determines the wave length of the transmission bands. The films are deposited by vacuum evaporation. A wave length λ_0 is transmitted when the distance between the reflecting

* The half-width is defined as the wave-length interval between the points on either side of the transmission band at which the transmission is half the maximum.

surfaces is $\lambda_0/2n$, where n is the refractive index of the dielectric. Besides maxima at frequency $\bar{\nu}_0$ corresponding to λ_0 , transmission maxima at all integral multiples of $\bar{\nu}_0$ are present.²² The maximum transmission of these narrow-band filters is about 30 to 40%, and the minimum transmission between maxima can be considerably less than 1%, but not, in principle, zero.

In converging or diverging light, the breadth of the transmission band is greater than in parallel light. If the filter is rotated so that the light does not pass through it normally the transmission band shifts to shorter wave lengths and splits into two with a wave-length separation increasing with the angle of incidence to the normal.²² The light in these separated transmission bands is polarized, the direction of vibration of the electric vector in the shorter component being parallel to the plane containing the normal and the incident ray, that of the other at right angles to this plane. It is therefore possible to isolate a monochromatic line from a spectrum, for example, the mercury spectrum, by a transmission-interference filter whose normal transmission band is at a longer wave length than the desired line, by tilting the filter in the beam into one of two positions until the line is transmitted. The transmitted light, however, will then be polarized. In using these filters in photometry it is therefore necessary to place the filter always accurately normal to the direction of the incident beam, for transmission of the nominal wave lengths, or reproducibly tilted for transmission of an adjusted wave length.

A filter designed for transmission in the blue, for example, at 4000 Å., may also have a transmission band in the red, if the thickness of the dielectric causes the "fundamental" band to be at 1.2 μ , whose second and third "harmonics" are at 6000 and 4000 Å., respectively. It is then necessary to combine the interference filter with another, usually a glass or dye filter, with high absorption for the undesired band and high transmission for the desired band. Interference filters of this kind are procurable commercially, for example, from *Baird Associates*, Cambridge, Mass., or *Farrand Optical Co., Inc.*, Bronx Blvd., New York 66, N. Y.

Another type of interference filter, by means of which the isolated light is *reflected*, consists of a perfectly reflecting mirror, on which is placed a thin layer of dielectric of thickness $\lambda_0/4n$, where n is the refractive index of the dielectric, covered by a very thin semiconducting layer of metal.^{22, 23} Practically complete transmission is effected by this filter for the frequencies $2\bar{\nu}_0$, $4\bar{\nu}_0$, $6\bar{\nu}_0$, etc., and zero transmission at $\bar{\nu}_0$, $3\bar{\nu}_0$, $5\bar{\nu}_0$, etc., where $\bar{\nu}_0$ is the frequency corresponding to λ_0 . The transmission bands, however, are broad.

²² L. N. Hadley and D. M. Dennison, *J. Optical Soc. Am.*, **37**, 451 (1947).

²³ L. N. Hadley and D. M. Dennison, *Phys. Rev.*, **69**, 258 (1946).

Fish-Schurman Corp., 230 East 45th St., New York, N. Y., supplies multilayer interference films, under the name of *dichroic* and *achromatic beam splitters*, which contain no metallic film. The dichroic films have high reflectance for light of one spectral region and high transmittance for the remainder of the spectrum; the breadth of the reflected and transmitted bands is relatively great. Achromatic or neutral beam splitters reflect one portion and transmit another portion of an incident beam without modifying its spectral character.

Glass and gelatin filters, and the cells for liquid filters, should have the care appropriate to apparatus of precision. When not in use, they are conveniently kept in a closed box with grooves cut to hold them vertically. The cleaning of the glass plates between which gelatin filters are mounted should be particularly carefully performed with the object of preventing damage to the gelatin by seepage of the cleaning liquid.

4. Photoelectric Photometers for Ultraviolet

An obvious extension of photoelectric photometry to the ultraviolet has been rendered relatively simple by the availability of inexpensive ultraviolet sources and ultraviolet-transmitting glasses. Substances with absorption bands at 2537 Å. can be analyzed by means of a light source such as the General Electric T-10 germicidal lamp,^{24, 25} most of whose radiation is emitted at that wave length (see Chapter XXI, page 1281). The Corning ultraviolet-transmitting glass 791, which passes about 50% of the radiation at 2500 Å., can be used as cell windows, or, preferably with organic solvents, an all quartz cell can be used. The RCA C 7032 phototube is a convenient receiver. Nothing new in principle is involved over the discussions of photoelectric photometry in the visible and reference need merely be made to some practical assemblies, such as that of Klotz,²⁵ for the analysis of liquids, such as solutions of benzene derivatives, sulfanilamide, and potassium nitrate, which absorb about 2537 Å., or of Hanson,²⁴ for the determination of the concentration of organic vapors in air, or of Silverman²⁶ for carbon disulfide vapor in air.

5. Photoelectric Colorimeters Using Optical Balancing

It is obviously possible to use photoelectric cells as a substitute for the eye in the balance method of divided-beam colorimetry exemplified in the visual Duboscq colorimeter (page 1435). A single source is caused to furnish two beams of identical intensity which pass, respectively, through two

²⁴ V. F. Hanson, *Ind. Eng. Chem., Anal. Ed.*, **13**, 119 (1941).

²⁵ I. M. Klotz, *Ind. Eng. Chem., Anal. Ed.*, **15**, 277 (1943).

²⁶ S. Silverman *Ind. Eng. Chem., Anal. Ed.*, **15**, 592 (1943).

cells containing the sample and a standard of the same color. The exit beams fall on two matched photocells in an electrically balanced circuit, and the length of one column of liquid is adjusted until the galvanometer deflection is zero, when equality of transmission by the standard and sample is indicated. Visual colorimeters, having optical systems designed for use with the eye, are not simply converted into efficient photoelectric instruments by mere introduction of photocells at the eyepiece, but satisfactory instruments can be made with the requisite optical changes. The Klett Photoelectric Duboseq Colorimeter is an example of this type of instrument, using photovoltaic cells.

IV. THERMOPILE AND BOLOMETER RADIOMETERS

Kipp and Zonen, Delft, Holland, have introduced a colorimeter and nephelometer using thermopiles and a sensitive low-resistance galvanometer in a balanced circuit. Since thermopiles are spectrally nearly non-selective, and incandescent sources emit most of their radiation in the infrared, special care is necessary in the selection of filters for use with these receivers to avoid unwanted infrared transmission bands.

Various improvements over the commercial instrument are discussed by Willard and Ayres.²⁷ On the whole there seems to be no particular advantage of thermopiles over photocells in the visible, ultraviolet and near-infrared, although for substances whose absorption is most readily measured in the infrared beyond the reach of suitable photoelectric cells they are the most convenient radiation meter. The possibility exists of developing methods of filter photometry in the infrared by means of thermopiles or bolometers bearing the same relation to the spectrophotometric methods at present being used in that region (Chapter XXI, page 1384) as the filter methods discussed in this chapter bear to visible and ultraviolet spectrophotometric methods of analysis.

An ingenious method of analysis of gases depending on their infrared absorption was introduced by Pfund.^{28, 28a} If the gas has an infrared absorption band it will, on being heated, emit selectively nearly the same radiation. A mass of the heated gas can therefore serve as a source whose radiation is absorbed specifically by the same gas.

Varying the method, an incandescent filament was used as source, and, as receiver, a thermopile with a polished receiver screened from the direct action of the radiation, mounted in a vessel containing the same gas as that to be measured, for example, carbon dioxide. In the absence of

²⁷ H. H. Willard and G. H. Ayres, *Ind. Eng. Chem., Anal. Ed.*, **12**, 287 (1940).

²⁸ A. H. Pfund, *Science*, **90**, 326 (1939).

^{28a} W. G. Fastie and A. H. Pfund, *J. Optical Soc. Am.*, **37**, 762 (1947).

carbon dioxide in an absorbing vessel between the source and receiver, the thermopile responds to the rise in temperature produced by the absorption of radiation in its atmosphere of carbon dioxide. The response is smaller if carbon dioxide is present in the absorption cell. High selectivity is attained in this way without the use of a spectrometer. Hydrocarbons, carbon dioxide, ammonia, the hydrogen halides, and many other gases would be amenable to analysis in this way.

Nonspectroscopic infrared analysis was developed considerably during the war. Representative of the apparatus is that of Wright and Herscher, sketched in figure 16, and used for the analysis of butadiene and styrene plant streams.²⁹ The method can be applied to many similar analyses.

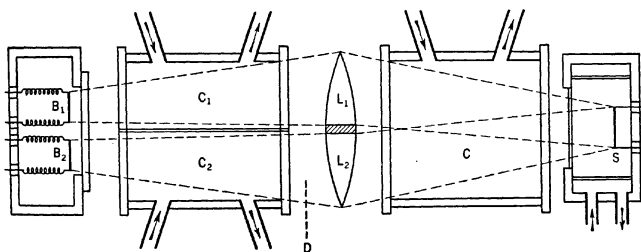


Fig. 16.—Infrared analyzer of Wright and Herscher.²⁹

Infrared radiation from an electrically heated Nichrome coil, *S*, mounted in a water-cooled housing (Fig. 16) is split into two beams by the half lenses, *L*₁ and *L*₂, which form images on the two nickel wire bolometers, *B*₁ and *B*₂ (page 1351). The bolometers form opposed arms of a Wheatstone bridge, and therefore equalizing the two beams by adjusting the movable stop, *D*, eliminates the effect of variation of the source. Any subsequent unbalance produced by absorption in one of the cells *C*, *C*₁, or *C*₂ is amplified by means of an electronic amplifier to actuate a potentiometer which automatically restores the balance of the Wheatstone bridge, and at the same time records the analysis, so that a continuous record of the composition of the stream can be obtained. *L*₁ and *L*₂ are made of rock salt or silver chloride, and windows are of lithium fluoride, rock salt, or silver chloride, according to the wave-length region absorbed by the substance to be introduced into the absorption cells (page 1343). A suitably placed split concave mirror can be substituted for the lenses. Pfund's method²⁸ of making the receivers selective for desired wave lengths can be used; the bolometer wires are embedded in a selectively absorbing medium which has strong absorption

²⁹ N. Wright and L. W. Herscher, *J. Optical Soc. Am.*, **36**, 195 (1946).

bands coinciding with characteristic bands of the component of the mixture to be determined, and no strong bands matching those of the other components.

As an example, consider the analysis of the total butenes in butadiene gas. In the region from about 1 to 4.7 μ butadiene has a strong single band at 3.27 μ , and each of the butenes (butene-1, *cis*- and *trans*-butene-2, and isobutene) has in addition a strong band centered at 3.4 μ . The analysis therefore depends on a measurement of absorption at 3.4 μ . The spectral range of the radiation entering the absorption cells is first restricted to the region 1 to 4.7 μ by operating the source at 800° C., at which temperature the maximum energy occurs at 2.7 μ , and by using a quartz exit window for the radiation leaving the housing. The bolometers are made selective to 3.4 μ by placing them in intimate contact with a film of ethyl cellulose, which absorbs this radiation strongly, but not 3.27 μ . A sample of the butadiene-butene mixture is placed in cell C_1 and the two beams balanced by adjustment of the stop D . If now a new sample with more of the butenes is placed in C_1 , less radiation will reach the bolometer B_1 and the amplifier will record a reading which, after calibration, will indicate the composition of the mixture.

In another variation, the sample is placed in C_1 , a pure specimen of the compound to be determined is placed in C_1 , and C_2 remains empty. Other examples of analysis are furnished in the paper of Wright and Herscher, and applications to continuous plant control and to automatic control of distillation are described. Instruments of this type are manufactured by *Baird Associates*, Cambridge, Mass.

A rapid infrared gas analyzer employing a thermistor (page 1352) as the receiver, capable of measuring the composition of very small samples, has been described by Fowler.^{29a} This apparatus is useful in physiological and other studies requiring measurements of rapidly changing gas concentrations.

V. SUBJECTIVE ABSOLUTE COLORIMETRY

Photometric measurements in the visible can also be made by visual methods of the kind already discussed in the section on visual spectrophotometry (Chapter XXI, page 1300). A single field of view, illuminated by two equally intense incident filtered beams, one passing through the solvent, the other through the sample, can be visually equalized by introducing a neutral wedge, by adjusting a calibrated aperture, by rotating a polarizing prism, etc., the transmission of the solution being read from the setting of the equalizing device at photometric balance. A color-

^{29a} R. C. Fowler, *Rev. Sci. Instruments*, 20, 175 (1949).

imeter using a wedge for equalizing the fields is made by *American Instrument Co.*, Silver Spring, Md.

Pulfrich Photometer

The Pulfrich Photometer, introduced by Carl Zeiss, is probably the best known of the subjective aperture photometers. In figure 17, light from a source, *S*, is divided into two equivalent parallel beams, which pass through cells *C* containing the solvent and sample, and are then brought together by a rhomb and by prism arrangement, *R* and *B*, so that an eye observing through eyepiece *E* perceives a photometric field divided by a sharp line, the two halves illuminated by light that has passed, respectively, through

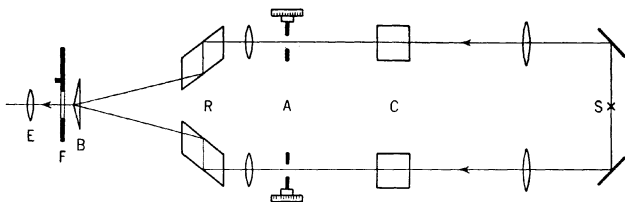


Fig. 17.—The Pulfrich photometer.

the solution and the solvent. The photometric adjustment is made by variable quadrilateral apertures, *A*, placed behind the absorption cells, the setting being made by means of drums graduated in per cent transmission and density (extinction). A set of filters, isolating narrow bands from an incandescent source or the monochromatic mercury lines, is mounted on disk *F*, which can be rotated so as to introduce any desired filter into the beam. Both tungsten and mercury lamps may be used, and the whole arrangement can be obtained solidly mounted on a bedplate, or can be assembled on an optical bench. Cell lengths from 1 to 50 mm. are available, using about 3.5 ml. of liquid per cm. length; and, for feebly absorbing substances, tubes 15 and 25 cm. long are provided.

Provision is made for equalizing the two beams when no absorbing substance is present, but absolute equality is not required if the measurement of absorption is made by first obtaining the setting for match with solvent and solution in the two beams, then repeating with the cells interchanged, and averaging the results. The eyepiece having been focused for sharpness of the dividing line of the field, the drum on the side of the sample is set for 100% transmission, and the other carefully rotated until balance is secured. This reading is repeated several times, with an equal number of approaches from higher and lower settings, and the operation repeated

with interchange of the cells. The average of all is taken as the transmission of the sample.

VI. MISCELLANEOUS TOPICS IN PHOTOMETRY

1. Multicomponent Systems

If two colored substances, each obeying Beer's law, are present in the same solution, their concentrations can be determined by measuring the absorption at two wave lengths, for which the respective molar extinction coefficients are known. Obviously if the absorption bands are so far apart in wave length as not to overlap appreciably, measurements with the use of sharp filters at the two maxima will permit the concentrations to be computed from equation (3) (see page 1403). If the bands overlap, measurements at two wave lengths at which the separate molar coefficients are markedly different in magnitude will permit the determination of the concentrations. If D' and D'' are the measured densities of substances 1 and 2 at wave lengths λ' and λ'' , c_1 and c_2 the concentrations of the absorbing substances, ϵ'_1 and ϵ''_1 the molar extinction coefficients of 1 at λ' and λ'' , ϵ'_2 and ϵ''_2 similarly the molar extinction coefficients of 2, and d the thickness of the absorbing layer, then

$$D' = (\epsilon'_1 c_1 + \epsilon'_2 c_2) d \quad (8)$$

$$D'' = (\epsilon''_1 c_1 + \epsilon''_2 c_2) d \quad (9)$$

from which:

$$c_1 = \frac{\epsilon''_2 \cdot D'/d - \epsilon'_2 \cdot D''/d}{\epsilon'_1 \epsilon''_2 - \epsilon''_1 \epsilon'_2} \quad (10)$$

and:

$$c_2 = \frac{\epsilon'_1 \cdot D'/d - \epsilon''_1 \cdot D''/d}{\epsilon'_1 \epsilon''_2 - \epsilon''_1 \epsilon'_2} \quad (11)$$

The bands transmitted by the filters for the isolation of the two wave-length regions should be narrow. Monochromatic mercury lines would be suitable.

2. Interference by Colored Substances and by Fluorescence

The effect of colored components other than that being determined can be compensated for in various ways. The effect of a colored solvent is almost completely eliminated if a substitution method is used in which the absorption of the solution is determined relative to that of the solvent.

Sometimes, the effect of interfering colored substances can be eliminated chemically, *e. g.*, in the determination of manganese by the formation of permanganate, Rowland²⁰ bleached the permanganate by adding hydrogen peroxide, after measuring the total absorption, the readings before and after addition of the peroxide permitting the computation of the absorption of the permanganate with respect to that of the colored background. Similarly, Sullivan and Norris²¹ determined the absorption of riboflavin in dried milk with reference to a colored background by reducing the riboflavin to the leuco compound. The latter authors also encountered the necessity of eliminating the effects of fluorescence of the substance under investigation. This was done by placing in front of the photocell a filter which absorbed much of the fluorescent light but transmitted light near the maximum of the absorption band. In certain cases, the effect of fluorescence can be largely eliminated by the addition of quenchers (page 1441). Precautions against fluorescence are particularly important when the sensitivity of the cell toward the fluorescent light is greater than that toward the absorbed light, as happens, for instance, in the use of the Photronic Cell with blue-absorbing substances which fluoresce in the green.

3. General Characteristics of Suitable Systems

The ideal colorimetric system for chemical analysis is one involving an intense absorption band in which, if the color is developed by the addition of reagents, the amount of absorption produced by a given amount of analysand reproducibly and rapidly attains its full and constant value. The value of the absorption, expressed in optical density, should preferably be proportional to the concentration of the analysand, and independent of the concentration of extraneous materials present, such as excess of the color-producing reagents and foreign salts. Rarely will the ideal be met, but one of the advantages of the absolute photometric methods of colorimetry is that the exact behavior of the system can be easily investigated and the precautions and corrections found which may make the system one of known degree of reliability. Such an examination should preface any use of a new colorimetric method.

The first essential in the development of a method of photometric analysis, after the discovery of the existence of a suitable color or the production of one by the addition of reagents, is the determination of the position and intensity of the absorption band. The most elaborate procedure is to find the spectrophotometric curve (Chapter XXI, page 1295), but in the absence of the equipment necessary for this, useful information can be ob-

²⁰ G. P. Rowland, Jr., *Ind. Eng. Chem., Anal. Ed.*, 11, 442 (1939).

²¹ R. A. Sullivan and L. C. Norris, *Ind. Eng. Chem., Anal. Ed.*, 11, 53 (1939).

tained from a mere spectrometric examination by instruments of the power of a hand spectroscope upward. Knowledge of the position of maximum absorption permits selection of the best filter (which should also be checked spectroscopically); and the intensity of the band determines the sensitivity of the method.

The colored system is then placed or produced in the absorption cell of the photometer, and rate of production and stability of the color found for various concentrations of the analytical material by noting any time dependence of absorption. The effect of added salts and other materials likely to be present in various amounts in the analytical material is also investigated. If a time dependence of the intensity of color is found, the method may nevertheless be usable if reproducibility is found after the lapse of a standardized time from the instant of mixing. Susceptibility to the influences of foreign substances may in some cases be corrected by the deliberate addition to the system of a considerably greater amount of the disturbing material than is likely to occur in the analytical material. Every disturbing influence must be considered as to its effects, and the accuracy of the analysis will fall with the number of the disturbances; but in systems by no means ideal standardized procedures of analytical worth can often be evolved. After preliminary observations have suggested the detailed procedure, the calibration curve of density *vs.* concentration is determined.

Colloidal suspensions are sometimes amenable to precise colorimetric analysis. They are likely to show time dependence of the absorption and to exhibit sensitivity toward electrolytes, which can sometimes be reduced by adding a protective colloid like gelatin.

VII. COMPARATIVE COLORIMETRY

1. Standard-Series Methods

In general, there are two principles of comparative colorimetry. (a) A match of the colors of an unknown sample and a known standard as viewed through the same thickness is made. If the standard and unknown are chemically identical, and prepared at the same time in the same solvents, then matching, with the proper optical disposition, indicates equality of concentration without further assumption. It is often convenient to use an arbitrary color standard of the same hue as the sample, for instance, a tinted glass disk; the stability of the standard is then a factor in the accuracy of the result. (b) The intensities of color of unequal thicknesses of standard and sample are balanced against each other. If Beer's law is obeyed, the concentrations of sample and standard are inversely proportional to the thicknesses for match; otherwise an empirical calibration by

means of standards of different concentrations must be made. The "balancing method" is the quickest and most convenient one of comparative colorimetry, and with instruments of the Duboseq type can be made of high precision.

The methods under (a) usually require very simple apparatus. Among them are:

(1) One or other of a colored sample or standard is diluted in vessels of the same diameter until the two colors match when viewed along the diameters of the tubes held side by side.

(2) Colorimetric titration, in which the color is produced in the sample by adding the necessary reagent, and a known volume of known concentration of the material of analytical interest is run into a blank containing the color-producing reagent until match is obtained with the sample. The volume of the blank before the addition of the standard is chosen slightly less than that of the colored sample, the standard is chosen relatively concentrated, and the final dropwise addition of standard is performed after the volume of the blank has been made equal, by the addition of solvent, to that of the sample.

(3) Standard-series methods. The simplest variation of the standard-series method is to make a set of standards of different concentrations of the substance in Nessler tubes mounted in a rack, and to seek to match the sample, observed axially against a white background such as a white card held at about 45° illuminated by light from the sky. A match having been found, it is well to see that it is maintained on interchanging the positions of the matching tubes. In more elaborate arrangements, the standards are placed on a rotating support situated so that the light transmitted by the sample and the adjacent standard are brought by a mirror and lens system at the exit end of the tubes to a single photometric field, one half illuminated with light from the sample, the other from the standard.

Nessler tubes with plane-parallel fused-on bottoms are now available from supply houses, and are an improvement over the forms with flattened bottoms ground and polished on the outside only.

The *glass-disk comparator* is a form of standard-series device especially useful when portability is desired. It is used mostly for pH measurements, but is also available commercially for the determination of small amounts of ammonia, nitrite, nitrate, chlorine, iron, and dissolved oxygen, as in water analysis. The Hellige Disc Comparator is a compact form for hydrogen-ion measurements, in which glass standards, made to match various indicator colors at specific electrometrically checked pH values, are mounted on a revolving disk. The solution under test, contained in a rectangular test tube, is observed through the disk, and another portion, containing the appropriate indicator in a definite amount, is observed di-

rectly. The disk is rotated until a match is found between a standard glass disk and the indicator color, the pH being read off from the disk. In one form of apparatus the two fields are brought into juxtaposition to increase the precision of comparison. The whole apparatus is small and easily portable. Disks are available covering the pH range from 0.2 to 13.6 at intervals of 0.2 unit, each disk containing nine standards. For small quantities of chlorine, oxygen, etc., in water, Nessler tubes 25 cm. long are used.

Similar in its use to the disk comparator but not readily portable is the *Lovibond tintometer*, long used in the specification of the colors of oils, spir-

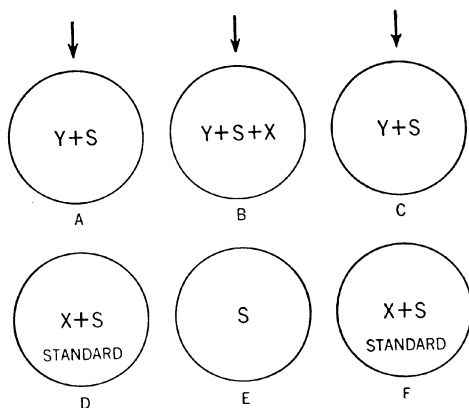


Fig. 18.—Walpole's arrangement for compensation for color.

its, etc. A large number of colored glasses is used, graduated in an arbitrary scale in terms of depth of color, and a match is sought between the specimen and some combination of glasses viewed in a suitable viewing box. The instrument has recently been used in the analysis of vitamins.

Another simple and well-known form of standard-series comparator is the *block comparator* used especially for hydrogen-ion determinations. Here the color standards are sealed-off test tubes containing buffered solutions at definite values of pH , usually differing by 0.2 unit, to which a definite amount of indicator has been added. The comparator is a block of wood containing a number of holes for the reception of the standards. Perpendicular to three of these holes are bored three observation holes. Three graduated tubes filled to the mark with the unknown solution are placed in holes A, B, and C (see figure 18); tubes containing known standards along with the color-producing agent are placed in holes D and F, and a tube containing solvent in E. The color is developed in the middle sample

tube (*B*) and the colored standards in *D* and *F* are selected so that, on looking through the tubes toward the light, a match of the middle pair with either of the others is secured, or so that the color of the middle lies between those of the lateral pairs. This arrangement, devised by G. S. Walpole, compensates for color or turbidity in the solution being measured, as can be seen from figure 18, which illustrates the situation when a color, *X*, to be compared with a standard is developed in a colorless solvent, *S*, containing a solution originally colored *Y*.

2. Balancing Methods

The simplest method of balancing the color of a sample to equality with that of a standard is to place the two solutions in separate, equally illuminated, flat-bottomed tubes and then to add the less intensely colored solution until the columns, when they are viewed axially from the top of the tubes, appear of equal intensity of color. Equality of illumination is checked by noting that equal columns of the same colored solution in the two tubes give equal intensity when viewed axially. If Beer's law holds for the system, the concentrations of sample and standard are inversely proportional to their lengths of column. In practice, the concentrations of sample and standard should not differ excessively to insure that both lengths are accurately measurable; also, the chance that the system will follow Beer's law is greater over a small range of concentrations than over a large range.

Hehner cylinders, which provide the measuring tubes with stopcocks near the flat bottoms for the withdrawal of the more intensely colored liquid until balance is achieved, add a certain measure of convenience to the operation of balancing; but, for precise analysis, a colorimeter allowing easy mechanical adjustment of the column lengths and employing the principle of juxtaposed fields is required. The modern forms of *Duboscq colorimeter*, models of which are made by many of the optical instrument makers of America and abroad, fulfill these requirements in instruments that are probably the most widely used of the subjective colorimeters. In figure 19, diffuse daylight or light from a frosted electric lamp is reflected from a mirror, *M*, or from a pair of independently adjustable mirrors, so as to traverse axially tubes *A* and *B* containing the colored standard and sample. The concentrations of standard and sample should be about the same. These tubes are mounted on holders movable up and down by manipulating a rack and pinion device. Into the tubes pass transparent plungers, rigidly mounted to the frame of the instrument, so that the length of the absorbing columns in cups *A* and *B* is determined by the positions of the bottoms of the plungers with respect to the bottom of the cups. The beams of light

transmitted by the solution are brought together by a prism arrangement, and an eye placed at eyepiece *E* sees a circular field, divided by a sharp line (the image of the line at which the prisms touch), one-half of which is illuminated by light which has traversed the solution in *A*, the other half by light which has traversed the other solution. The walls of the cups and

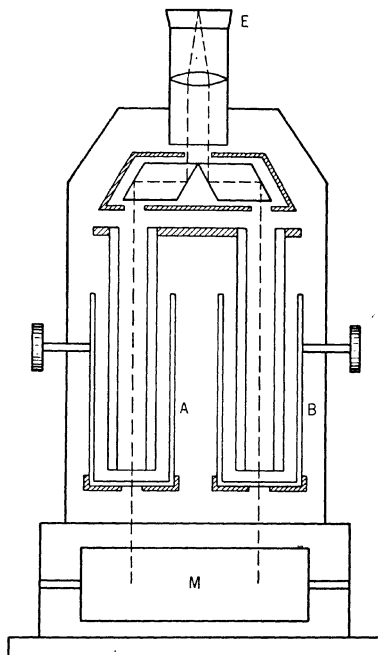


Fig. 19.—Dubosq colorimeter.

plungers are sometimes made of black glass. The position of the bottom of each plunger with respect to the bottom of its cup is indicated by a scale and vernier, the scale adjustable so that it reads zero when the plunger and cup are in contact. Cups should not be interchanged after this adjustment has been made.

In using the instrument the first operation after adjustment of the scales is to ascertain that equality of illumination of the two cups exists. Standard solution is placed in both cups and the scales are set to the same read-

ing. The two halves of the field should appear equally bright; if not, the position of the source and mirrors is adjusted until this is so. The sample is added to one cup, the depth of the standard column adjusted to a definite value, and the cup containing the sample adjusted until match is secured in the field.

It may happen that optical and mechanical imperfections in the instrument prevent a match at equal thicknesses of the same solution illuminated as described. In that case, a fixed solution, say the standard, can be kept in one of the cups, and all measurements of standard and sample made in the other cup with respect to the fixed solution. If S is the position of balance for the standard in the measuring cup, and X that of the sample in the same cup, both balanced against the fixed solution, the concentration of the sample is S/X that of the standard, provided Beer's law is true.

Microcolorimeters are available which use 1 ml. of liquid in the cups.

Color filters are not generally used in conjunction with the Duboscq colorimeter. Weigert³² claims an increase in the accuracy of setting for match by the use of filters. For instance, in the matching of two blue solutions, the introduction of a yellow filter so as to make the transmitted light as nearly as possible a neutral green causes color differences as well as intensity differences until balance is achieved, there being more yellow in the light transmitted by the variable column when it is too thin and more blue when it is too thick.

The conventional balancing methods cannot be used without modification in colorimetric systems involving equilibrium between two or more differently colored components, because of the changes in hue that accompany the variation of the length of the absorbing columns. The determination of the concentration of hydrogen ions by means of two-color indicators is a case in point. If the indicator acts as a simple weak acid dissociating into a differently colored ion with an apparent dissociation constant, K_a ,³³

$$pH = pK_a + \log \frac{\alpha}{1 - \alpha} \quad (12)$$

where α is the degree of dissociation of the acid. If the two forms absorb light independently, it should be possible to match the color transmitted by a sample which confers on the indicator a color between those of its acid and alkaline forms by a column of indicator of the same total thickness and

³² F. Weigert, *Optische Methoden der Chemie*. Akadem. Verlagsgesellschaft, Leipzig, 1927, p. 249.

³³ W. M. Clark, *The Determination of Hydrogen Ions*. 3rd ed., Williams & Wilkins, Baltimore, 1928, p. 121.

concentration, divided in the appropriate ratio into separate columns of pure acid and pure alkaline forms. The thickness of the alkaline layer is a measure of α and that of the acid layer of $1 - \alpha$. If pK for the indicator is known, the pH of the sample can be computed, or read from tables.³⁴

The addition of an extra cup to the ordinary arrangement of the Duboseq instrument permits this measurement to be made, as in the Bausch and Lomb Hydrogen Ion Colorimeter. In figure 20, the sample plus indicator is placed in the right-hand cup, C_1 . In the left cup, C_2 , is placed an indicator solution of the same concentration as in the sample, completely converted to the alkaline form by the addition of sodium hydroxide. The

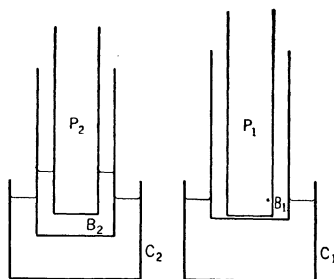


Fig. 20.—Principle of the hydrogen-ion colorimeter.

movable cup, B_2 , is provided with a similar solution of the acid form, while the empty cup, B_1 , is placed in contact with plunger P_1 . The bottoms of C_1 and C_2 are placed the same distance, say 20 mm., from the bottoms of their respective plungers, so that the total optical path through the liquids in the two limbs is the same. The position of B_2 is varied until the light transmitted by the acid and alkaline forms matches that transmitted by the sample.

The Bausch and Lomb instrument is provided with auxiliary cups which can be placed below the measuring cups, allowing the use of Walpole's method (page 1434) for compensating for initial color or turbidity in the sample.

The principle of this measurement can be applied to the matching of a mixture of any two suitable independent colors by an equal total thickness of the separate constituents.

³⁴ For example, in F. D. Snell and C. T. Snell, *Colorimetric Methods of Analysis*, Vol. I, Van Nostrand, New York, 1936, p. 704.

VIII. FLUORIMETRY*

1. Characteristics of Fluorescence

The intensity of the light emitted by a fluorescent solution is, under certain circumstances, a simple function of the concentration, and can therefore be used for analysis. The phenomenon has long been used for "spot test" examination of drugs, etc.; and more recently fluorescence methods have become important in the determination of thiamine and riboflavin.

The sensitivity of fluorescence methods may exceed that of colorimetric methods. For example, a standard colorimetric method for the estimation of thiamine requires 2 to 3 $\mu\text{g.}$ per cc., while 0.05 $\mu\text{g.}$ per cc. can be measured in terms of the fluorescence of its oxidation product, thiachrome.³⁵ Riboflavin at a concentration of 0.0005 $\mu\text{g.}$ per cc. can be detected colorimetrically.³⁶

The nature of fluorescence has been outlined in Chapter XXI (page 1248) and only some additional aspects of importance in analysis will be mentioned here. With the rare exceptions of the "resonance fluorescence" of certain dyes in the mesomorphic state,³⁷ the light emitted by fluorescent solutions has its maximum intensity at a wave length longer than that of the exciting absorption band, usually by some two or three hundred angstroms. Fluorescent substances with visible absorption bands frequently possess absorption bands in the ultraviolet, excitation in which usually produces the same fluorescence spectrum as does excitation in the visible. There may sometimes be an advantage in the use of ultraviolet light in such a case, in visual observation, because of the elimination of the effect of spurious exciting light mixed with the fluorescence, but often short-wave-length exciting light causes photochemical destruction of the substance at a greater rate than light of longer wave length.

The general scheme in fluorimetric measurements is therefore to excite with radiation at the wave length of maximum absorption, isolated by filters from a source such as a mercury or incandescent lamp, and to measure or compare with that of some standard the intensity of fluorescent light, carefully freed from reflected incident light by passage through a filter or monochromator. The measurements are essentially similar to those al-

* A review of recent methods in fluorimetric analysis is given by C. E. White in *Anal. Chem.*, **21**, 104 (1949).

³⁵ R. A. Brown, E. Hartzlen, G. Peacock, and A. D. Emmett, *Ind. Eng. Chem., Anal. Ed.*, **15**, 4946 (1943).

³⁶ F. Kavanagh, *Ind. Eng. Chem., Anal. Ed.*, **13**, 108 (1941).

³⁷ E. E. Jelley, *Nature*, **138**, 1009 (1936). G. Scheibe, L. Kandler, and H. Ecker, *Naturwissenschaften*, **25**, 75 (1937).

ready described for colorimetry, and the instruments used are identical with, or simple adaptations of, colorimetric instruments.

At low concentrations, the *intensity* of fluorescence is proportional to the intensity of absorbed light, that is, the ratio of the fluorescent to the absorbed light, or fluorescence efficiency, is independent of the concentration. This efficiency, in terms of the number of quanta of fluorescent to absorbed light, may vary from nearly one in strongly fluorescing substances like fluorescein at very low concentration to any lower value. As the concentration increases, however, the efficiency falls. The concentration at which this "concentration quenching" sets in varies with the substance, but may be as low as 10^{-4} mole per liter. Its origin is probably complex, including quantum mechanical resonance interactions between excited and unexcited molecules as well as changes in the molecular species resulting from association at higher concentrations.

Proportionality between the intensity of fluorescence and concentration can exist therefore over only a very limited range within the region of constant efficiency and at absorptions small enough to be approximately proportional to the concentration. Below about 20% absorption (density = 0.1), the absorption is proportional to the concentration with a maximum deviation of about 10% of the value of the absorption, if Beer's law is true, and the corresponding concentration can be found, given the molar extinction coefficient, from the equation $c = D/\epsilon d$ (page 1401). Thus, if ϵ is 5000 liter mole⁻¹ cm.⁻¹ and d is 5 mm., the concentration below which the fluorescence intensity is proportional to the concentration within 10% is $c = 0.1/5000 \times 0.5 = 4 \times 10^{-6}$ molar, provided concentration quenching and instrumental characteristics do not intervene.

In practice, the range of concentrations approximately proportional to the observed fluorescent intensities depends on the instrumental details. In visual observation, it will be greater if the fluorescent light is observed in the same direction as the incident light than perpendicular to the incident light, for, in the former case, change in the fluorescent intensity with increasing penetration of the exciting light into the solution does not interfere. For instance, measurements by B. Enneking³⁸ showed proportionality between concentration and fluorescence intensity in quinine bisulfate up to 0.1 g. per liter as observed in the Pulfrich photometer (page 1429) and only to 0.01 g. per liter in a nephelometer, with sidewise illumination. In the latter type of instrument, in which the field of view is illuminated by light from a section of the fluorescing volume in the interior of the cell, isolated by means of stops to prevent the entrance of spurious light into the field, complicated intensity-concentration relations may exist; and, at high con-

³⁸ Quoted in P. W. Danckwortt, *Lumineszenz-Analyse*. Akadem. Verlagsgesellschaft, Leipzig, 1934, p. 120.

centration, the intensity may not even be a single-valued function of the concentration.

Fluorescence may decrease or increase with *temperature*, depending on the substance, concentration, and temperature. The normal effect is a decrease with increasing temperature. Usually the variation with a few degrees is sufficiently small not to require thermostating, but care should be taken to avoid gross temperature changes, for instance, due to heat from the light source.

The fluorescence of many compounds depends on the *hydrogen-ion concentration*. Some are known which undergo a very large change in intensity over a relatively small range of *pH* and can therefore be used as indicators. It is possible to determine the dissociation constants of acids and bases if the fluorescence of the ion is markedly different from that of the undissociated molecule.³⁹ It is therefore important in the analysis of such substances to carry out the measurements at a *pH* at which the change of fluorescence with *pH* is small, or to use buffered solutions, or at least to keep the solutions in chemically inert bottles and measuring cells.

Besides showing concentration quenching and susceptibility to the effect of *pH*, many substances have their fluorescence *quenched by neutral salts and organic substances*, apparently chemically indifferent, and with absorption bands far removed from that of the luminescent material. Over a restricted range, the quenching is a hyperbolic function of the concentration of the quencher; if *I* is the intensity of fluorescence in the presence of concentration *c* of quencher and *I*₀ the intensity in the absence of quencher:

$$I/I_0 = 1/(1 + kc) \quad (13)$$

where *k* is a constant. In many cases, the quenching is due to the anion, ions with loosely bound electrons, as iodide and thiocyanate, being more powerful quenchers than those with tightly bound electrons, like fluoride and perchlorate. In other cases, oxidizing ions like nitrate have pronounced quenching effects. Of organic molecules, polyhydroxy phenols, amines, and, in general, "antioxidants" are often efficient quenchers. Although no permanent reaction between quencher and fluorescent substance occurs, the phenomenon seems often to depend on reversible oxidation-reduction reactions between the optically excited molecule and the quencher, followed by regeneration of the original substances. Fluorescent substances which are easily reduced to colorless products are likely to be quenched by hydroquinone and iodide ion, while more stable substances are likely to resist quenching by these substances.

The intensity of fluorescence is sometimes increased by the addition of

³⁹ L. J. Desha, R. Sherrill, and L. Harrison, *J. Am. Chem. Soc.*, **48**, 1493 (1926).

foreign ions, for example, the fluorescence of the uranyl ion is enhanced by addition of fluoride ion.⁴⁰ The susceptibility of a fluorescent substance to quenching or enhancement should therefore be ascertained before fluorimetric analysis is attempted, and if the effect is marked care must be taken in the control of the concentration of quencher in sample and standard.

The effect of quenchers on the fluorescence of substances of high susceptibility, as quinine bisulfate, may be used as a means of estimation of the concentration of the quencher.

2. Qualitative and Semiquantitative Fluorimetry

(a) Although not strictly an example of fluorimetry, fluorescence capillary analysis should be mentioned. As described by Danckwortt,⁴¹ strips 25 cm. long and 2 cm. wide are cut out of filter paper in a uniform direction with respect to the fiber. Schleicher and Schull No. 602 paper is recommended as nonfluorescent. A 5-ml. portion of the liquid under examination is placed in a cylinder 5 cm. high and 2.5 cm. wide, and a strip inserted so as to touch the bottom. The temperature is held between 18° and 20° C. and the humidity between 70 and 75%. The operation is conveniently carried out in a closed glass vessel, for example, in an old balance case, maintained at constant humidity. After 24 hours, during which the apparatus is protected from the light, the strip is removed and dried in air, in the dark. On examination in u.v. light (Hg arc with nickel oxide glass filter) (p. 1423), characteristic luminous zones are observed which can be compared visually with standards. As an example of the sensitivity of the method, 1 γ of morphine in 30 ml. of liquid is distinctly recognizable.

(b) The simplest way of comparing intensities of two fluorescing solutions in a semiquantitative way is to illuminate them side by side and to observe them through filters excluding the exciting light. Match of an unknown can be sought with one of a series of standards, or the standard and sample can be made of equal fluorescent intensity by diluting the one or the other.

3. Quantitative Fluorimetry

A. VISUAL METHODS

The *Pulfrich photometer* (page 1429) is convenient for the visual comparison of two fluorescing solutions. It can be used as in colorimetry, with light from a mercury or incandescent lamp, filtered to include only radiation absorbed by the solution, and with the interposition of filters in the fluorescent beam carefully chosen to transmit none of the exciting radiation.

⁴⁰ E. Jette and W. West, *Proc. Roy. Soc. London*, A121, 307 (1928).

⁴¹ P. W. Danckwortt, *op. cit.*, p. 21.

The less bright of the two luminous fields seen in the eyepiece is set at 100 on the transmission scale and the other equalized by partially closing its aperture. If it is known that standard and sample are in the concentration range of proportionality between fluorescent intensity and concentration, the ratio of the concentrations is directly read from the drum readings.

J. Eisenbrand⁴² has given equations permitting the determination of the relative concentrations of two fluorescent solutions at higher concentrations, provided that they are in the range of constant fluorescent efficiency, that none of the fluorescent light is absorbed by the solution, and that the absorption of the exciting light obeys Beer's law. These equations are valid for any method, visual or photoelectric, in which the direction of the fluorescent beam is the same as or nearly directly opposite to that of the exciting beam, but not when the fluorescent light is viewed by the receiver at right angles to the direction of the incident beam. In the former case the variation of the fluorescent intensity with the depth of penetration of the exciting beam is not an interfering factor, while in the latter it is. The two principles are that the intensity of fluorescence is directly proportional to the intensity of the absorbed light and that the concentration is directly proportional to the density of the absorbed light ($= \log I_0/I$ where I_0 and I are the intensities of the incident and transmitted exciting light before passage through any filter on the exit side of the cell). Consider a solution sufficiently concentrated for all of the exciting light to be absorbed within it, the concentration always being within the range of constant fluorescence efficiency. The fluorescence then excited, F_c , being proportional to the absorbed intensity, is a measure of the incident intensity I_0 , i. e., $I_0 = kF_c$. The fluorescence excited in a less concentrated solution is proportional to the absorbed intensity, $I_{\text{abs.}} = kF$. Now the density of absorption is $D = \log I_0/I_{\text{trans.}} = \log I_0/(I_0 - I_{\text{abs.}}) = \log F_c/(F_c - F)$, and according to Beer's law this quantity is proportional to the concentration. The ratio of two concentrations, c_1 and c_2 , can then be determined from their fluorescence intensities F_1 and F_2 relative to a solution in which all of the exciting light is absorbed, from the equation:

$$\frac{c_1}{c_2} = \frac{\log F_c - \log (F_c - F_1)}{\log F_c - \log (F_c - F_2)} \quad (14)$$

If the fluorescence of the solutions is measured as a percentage of that of the solution of complete absorption:

$$\frac{c_1}{c_2} = \frac{2 - \log (100 - F_1)}{2 - \log (100 - F_2)} \quad (15)$$

⁴² Quoted in P. W. Danckwortt, *op. cit.*, p. 123.

The *nephelometer*, that is, a Duboscq colorimeter in which the cells are illuminated from the sides, ordinarily used to compare turbidities, can also be used in fluorimetry. Provided the difference in concentration between the sample and the standard is not too great, the intensities of fluorescence are inversely proportional to the lengths of the illuminated columns at match. Details may be found in an article by Desha.⁴³

The *Duboscq colorimeter*, with longitudinal illumination, has also been used in fluorescence analysis, but is not very well suited because the relative intensities are not inversely proportional to the lengths of column at match.

B. PHOTOELECTRIC METHODS

As in colorimetric analysis, there has recently been considerable activity in the development of photoelectric fluorimeters. Indeed, the same instrument can often be used for both purposes. Both photovoltaic and emissive cells have been used; with the former, the low intensity of the fluorescent light makes the use of a low resistance galvanometer of high sensitivity desirable (about 5×10^{-9} amp. per division), while vacuum-tube amplification of the currents from the emissive type permits the use of a less sensitive instrument. Electron multiplier tubes can also be used. It is particularly important in photoelectric measurement to exclude the entry of any of the exciting radiation into the receiver, as it lacks the ability of the eye to recognize spurious light of different color from the fluorescent light. Direct deflection, differential and null circuits can be used, of which the null ones are the best (page 1417). Compensation can be accomplished by the methods mentioned in the discussion of photoelectric colorimetry, for instance, by the use of a variable aperture, a calibrated neutral wedge, or potentiometrically. The circuit of Brice (Fig. 11, page 1418) is well adapted to fluorimetric measurements, potentiometric balancing of the unknown being made against a standard fluorescent solution or against an arbitrary standard.

Figure 21 shows diagrammatically the simplest kind of direct-deflection photoelectric fluorimeter. The fluorescent material, in cell *A*, is illuminated by light from a source, *S*, for example, a mercury lamp of the H-4 type set in a ventilated housing. Filter *F*₁ transmits only light absorbed by the sample. The cell is placed in a blackened holder provided with apertures for the entrance of the exciting light and exit of the fluorescent light toward the photocell, *B*. The same windows are always presented toward the source and phototube, respectively. An amplified photoemissive cell is represented in the diagram, but a photovoltaic cell and sensitive galvanometer could also be used. Filter *F*₂ is chosen so as to transmit only the

⁴³ L. J. Desha, *J. Am. Chem. Soc.*, **42**, 1350 (1920).

fluorescent light, minimizing scattered and reflected exciting light. The photocell is connected to an amplifier, *C*, in the usual way (page 1317). Details of a fluorimeter of this type and an amplifying circuit supplied from the mains are given by Krebs and Kersten.⁴⁴

The readings of a fluorimeter of this kind consist of galvanometer or microammeter deflections in the output of the amplifier. In multitube feedback amplifiers, such as those of Krebs and Kersten (compare also page 1319), readings are made on a microammeter, while in single electrometer tube amplification, as in the circuit of DuBridge and Brown, a fairly sensitive mirror galvanometer is required.

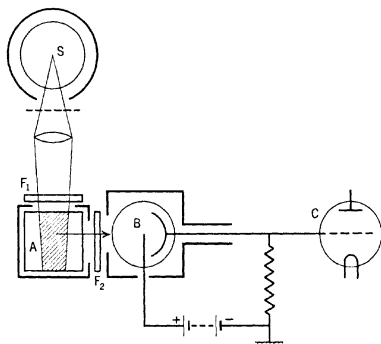


Fig. 21.—Simple fluorimeter.

There will always be some deflection of the meter when a nonfluorescent liquid is illuminated in the cell. A reading must therefore be obtained for the solvent and subtracted from that obtained for the sample, or the meter may be set to zero for the condition in which the cell contains illuminated solvent. If the fluorescent substance can be quenched or chemically removed without introducing disturbing factors, the zero correction can be made by subtracting the reading for the quenched sample from that obtained before quenching.

Inconstancy of the source will often prevent the use of a simple calibration curve of meter deflections plotted against known concentrations of the fluorescent material. The best procedure is to express the intensity of fluorescence of the sample in terms of the readings of an easily reproducible standard, which may be the pure fluorescent substance under test, or another readily purified fluorescent substance with absorption and fluores-

⁴⁴ R. F. Krebs and J. Kersten, *Ind. Eng. Chem., Anal. Ed.*, 15, 132 (1943).

cence bands similar to those of the sample, or a fluorescent glass standard in the form of a block fitting onto the fluorescence cell.⁴⁵ Quinine in dilute sulfuric acid,⁴⁶ for example, is a convenient standard for use with thiochrome. If the deflections for a standard solution of quinine in dilute sulfuric acid, for a thiochrome extract prepared from n micrograms of thiamine, and for the solvent, isobutyl alcohol, all at a definite volume are, respectively, Q , T , and S , the deflection per microgram of thiamine can be expressed as $(T - S)/nQ$. The deflection for the unknown is taken as the difference in readings for the oxidized sample and the nonfluorescent sample before oxidation, in terms of the standard quinine readings, and the number of micrograms of thiamine in the sample in the measuring cell is this quantity divided by the deflection per microgram.

Peterson, Brady, and Shaw⁴⁷ use a slightly different procedure in calibrating for riboflavin. Into each of two cells, A and B , is pipetted a given volume, say 5 cc., of sample extract. To A is added 5 cc. of water, and to B 5 cc. of a standard riboflavin solution containing 0.2 $\mu\text{g.}$ per cc. The readings for A and B are taken, and the riboflavin in both solutions destroyed by reduction with sodium hydrosulfite. The average of the two readings after this procedure is taken as the blank, or C reading. Then: sample reading = $A - C$. Reading for sample + 1 $\mu\text{g.}$ in 10 cc. = $B - C$. Reading for 1 $\mu\text{g.}$ in 10 cc. = $B - C - (A - C) = B - A$. Number of $\mu\text{g.}$ per cc. of sample = $(A - C/B - C) \times 0.1$. These estimates assume linearity between fluorescent intensity and concentration of the sample material.

In analysis, the fluorescence measurement of a sample is preceded by a more or less extensive chemical treatment for the isolation and purification of the material. A blank should be made by carrying out the isolation procedure with all the reagents except the analytical material, and the fluorescence reading subtracted from that found for the sample. Filter paper of as low fluorescence as possible should be used (page 1442) and, instead of hydrocarbon stopcock grease for lubrication in any part of the apparatus, nonfluorescent materials such as silicone grease, glycerine, or phosphoric acid should be used.

By suitable separation, more than one fluorescent material in a mixture can be analyzed. Conner and Straub⁴⁸ describe a procedure for the analysis of thiamine and riboflavin from the same extract, after separation by selective adsorption and subsequent elution. In the determination of riboflavin

⁴⁵ E. Loewenstein, *Ind. Eng. Chem., Anal. Ed.*, **15**, 658 (1943).

⁴⁶ D. J. Hennessy and L. R. Cerecedo, *J. Am. Chem. Soc.*, **61**, 179 (1939).

⁴⁷ W. J. Peterson, D. E. Brady, and A. O. Shaw, *Ind. Eng. Chem., Anal. Ed.*, **15**, 639 (1943).

⁴⁸ R. T. Conner and G. J. Straub, *Ind. Eng. Chem., Anal. Ed.*, **13**, 380 (1941).

the Corning filter 511 was found suitable for the isolation of the incident light, and the Corning 351 for the fluorescent light.

Fluorescent organic substances may undergo photochemical change, and it is important in such cases to expose them for as short a time as possible. Since the lamp will require running for some minutes to attain adequate constancy in intensity, a shutter should be interposed between the source and fluorescence cell except during the measurement. In the determination of thiamine by the thiachrome method, Kavanagh³⁶ recommends making at least four readings in the first two minutes and extrapolating to zero time on a plot of the logarithm of the reading against time.

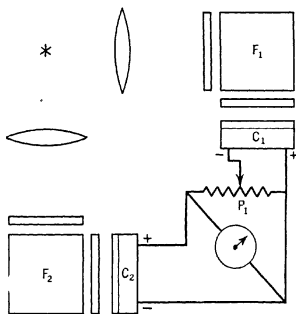


Fig. 22.—Two-cell fluorimeter.

In figure 22 a two-cell fluorimeter is shown employing the compensated circuit of Brice for photovoltaic cells (page 1418). The readings of the sample are expressed as the ratio to that of a standard, and effects of the inconstancy of the light source are eliminated. The arrangement of source, fluorescence cell, filters for exciting and fluorescent light, and shutters are the same as in figure 21. With the fluorescent standard, say a solution of quinine in dilute sulfuric acid for substances whose fluorescent light is blue, or sodium fluorescein for green fluorescers, in both cells F_1 and F_2 , potentiometer P_1 is set at 100, and the intensity of light falling on photocell C_2 is adjusted (by use of a variable diaphragm, or by tilting the cell to receive more or less of the fluorescent light) until the galvanometer reads zero. The sample, whose fluorescent intensity should be lower than that of the standard, is then placed in fluorescence cell F_2 . The galvanometer will deflect and the contact on the compensating potentiometer must be moved to a lower value to regain balance. If the conditions of Brice's circuit are fulfilled, the setting of the potentiometer will give the fluorescence intensity of the sample as a percentage of that of the standard. A sensitive

galvanometer of relatively low resistance should be used (about 10^{-9} amp. per mm.).

IX. TURBIDIMETRY

1. Introduction

The appearance of cloudiness in a suspension of small particles originates in the lateral scattering of light from the direction of the incident beam by particles whose refractive index differs from that of the suspension medium. Light is therefore observed from directions sidewise to the primary beam, and the intensity of the beam in the incident direction is attenuated as it penetrates the suspension, as if it were traversing an absorbing medium. There is no loss of light energy as such, and the weakening of the beam in the primary direction is sometimes referred to as "*conservative absorption*," in contradistinction to true or "*consumptive absorption*," in which light energy disappears by conversion into some other form.

The amount of scattered light increases with the number of scattering particles, and the phenomenon can therefore be made the basis of procedures in analytical chemistry. Since the introduction of the nephelometer ($\nu\epsilon\phi\epsilon\lambda\eta$, a cloud) by Richards,⁴⁹ methods depending on light scattering have enjoyed a well-known but restricted vogue in analysis and in specialized technological techniques such as the measurement of the clarity of water supplies or of industrially important filtrates. Within recent years, however, interest in the scattering of light has become more general among chemists, with the realization—largely prompted by Debye—that the phenomenon could yield information concerning the number, dimensions, and shapes of large molecules, which is of interest to the growing groups of chemists and biochemists concerned with high polymers of various kinds. The theory, much of whose fundamentals is contained in literature dating back to Rayleigh's studies in 1871, has been developed into forms suitable for the determination of molecular weights by Debye, which have been developed experimentally and theoretically largely by Debye and his co-workers and by Mark, Doty, and Zimm and their collaborators. Since it appeared that this aspect of the application of light scattering was more pertinent to the interests of organic chemists than purely analytical applications, most of this section has been devoted to an outline of the theory and a description of the experimental methods used by the workers in the field of high polymers. For a careful account of the analytical uses of subjective nephelometry, reference is made to Yoe's book.⁵⁰

In compiling this discussion of the application of light scattering to the

⁴⁹ T. W. Richards, *Proc. Am. Acad. Arts Sci.*, **30**, 385 (1894).

⁵⁰ J. H. Yoe, *Photometric Chemical Analysis*. Vol. II, Wiley, New York, 1929.

molecular-weight determination of polymers, the writer wishes to acknowledge the assistance he has derived from the comprehensive chapter on light scattering in polymer solutions, by Mark,⁵¹ which was made available to him before publication through the kindness of Professor Mark. In addition, frequent reference will be made to the following articles.⁵²⁻⁵⁹ A pioneering study on the determination of the molecular weights of proteins by light scattering was carried out by Putzeys and Brosteaux.⁶⁰

A note on nomenclature may be appropriate. In principle, measurements of light scattering may be made in terms of: (a) the light diverted from the primary beam, or (b) the diminution in intensity of the primary beam caused by the lateral scattering. In the literature of analytical chemistry the convention seems to have arisen of restricting the term turbidimetry to methods based on measurements in the direction of the primary beam, sometimes with the specific description "extinction turbidimetry," and to distinguish methods based on lateral scattering as nephelometry or tyndallometry according as the scattering is great enough to cause an obvious opalescence or not. Since, in the determination of molecular weights, the important quantity has been called the "turbidity," whether it is determined from measurements of the laterally scattered beam or of the primary beam, those interested in high polymers have designated as turbidimeters instruments designed both for the measurement of lateral scattering and of transmission—in fact, both types of measurement may be made by means of a single instrument. No great confusion seems likely to be caused by this use of the term.

2. Theory

A. IDEAL SOLUTIONS OF SMALL PARTICLES

If a beam of parallel light is passed through a transparent medium containing small particles whose refractive index differs from that of the rest of the medium, the particles act as centers for the propagation of light in all directions, with the result that the primary beam is weakened in intensity and light is emitted in side-wise directions. As is well known, in the wave theory of the rectilinear propagation of light through a transparent, perfectly homogeneous medium, the absence of

⁵¹ H. Mark, in *Chemical Architecture* (Frontiers in Chemistry, Vol. V). Interscience, New York, 1948.

⁵² P. Debye, *J. Phys. & Colloid Chem.*, **51**, 18 (1947).

⁵³ P. Doty, H. Wagner, and S. Singer, *J. Phys. & Colloid Chem.*, **51**, 32 (1947).

⁵⁴ P. M. Doty, H. Zimm, and H. Mark, *J. Chem. Phys.*, **13**, 159 (1945).

⁵⁵ R. S. Stein and P. Doty, *J. Am. Chem. Soc.*, **68**, 159 (1946).

⁵⁶ G. Oster, P. M. Doty, and H. Zimm, *J. Am. Chem. Soc.*, **69**, 1193 (1947).

⁵⁷ B. H. Zimm, *J. Phys. & Colloid Chem.*, **52**, 260 (1948).

⁵⁸ P. M. Doty, W. A. Affens, and B. H. Zimm, *Trans. Faraday Soc.*, **B42**, 66 (1946) (General Discussion of Swelling and Shrinking).

⁵⁹ V. K. LaMer, *J. Phys. & Colloid Chem.*, **52**, 65 (1948).

⁶⁰ P. Putzeys and J. Brosteaux *Trans. Faraday Soc.*, **31**, 1314 (1935).

radiation in directions lateral to that of the primary beam is due to the destructive interference, in all directions except that of the primary beam, of the secondary wavelets which may be considered as produced at each point in the medium; any inhomogeneity will upset this condition and produce lateral scattering. The intensity of a parallel primary beam is then attenuated in its passage through the inhomogeneous medium according to the equation:

$$I = I_0 e^{-\tau x} \quad (16)$$

where I is the intensity in the direction of the primary beam, of initial intensity I_0 , after traversing a distance x in the medium.* The coefficient τ , called the *turbidity* of the medium, plays the same part in the weakening of the primary beam by scattering as does the absorption coefficient in consumptive absorption. The dimensions of τ , like those of the absorption coefficient, are cm.^{-1} , and τ can be thought of as the reciprocal of the distance through which a parallel primary beam must penetrate the medium in order to be weakened by scattering to $1/e$ or 37% of its initial value. For polymer solutions at 1% concentration τ is of the order of 10^{-3} , that is, the primary beam is weakened to 37% of its incident value in a distance of the order of 10 meters, while the corresponding distance for a 1% solution of small molecules is about 100 meters, and for pure liquids about 1 kilometer.

The great bulk of the scattered light has the same wave length as the primary light, but a small fraction, of the order of 0.1%, is of modified wave length, and constitutes the Raman scattering (Chapter XXI, page 1371).

The turbidity, τ , in general, is a function of the wave length of the primary light, of the concentration, size, shape, and relative refractive index of the scattering particles, and of mutual solute-solute orientations and solvent-solute interactions. In the simplest case, that of independent particles with no dimension greater than $1/16$ to $1/10$ of the wave length of the primary light, and with no phase relations between the wavelets scattered by the different particles, each particle radiates as an independent dipole set into oscillation with the frequency of the light. The fraction of the incident light scattered in all directions by unit volume of the medium, equivalent to the fractional diminution of the intensity in the direction of the primary beam per unit length, the quantity denoted by τ in equation (1), is then given by:⁵²

$$\tau = \frac{8\pi}{3} \left(\frac{2\pi}{\lambda} \right)^4 n \alpha^2 \quad (17)$$

where n is the number of scattering particles per cc., λ the wave length of the incident light, and α the polarizability of the particle, i. e., the proportionality factor relating the magnitude of the induced electric moment to the strength of the exciting electric field. This equation is equivalent to the classic formula of Rayleigh, and scattering which conforms to this law is often called "Rayleigh scattering."

For spherical particles, α is proportional to the volume; and the intensity of the scattered light is therefore proportional to the square of the volume

* This is strictly true only when the centers of inhomogeneity are sufficiently dilute to make negligible any secondary scattering in the forward direction.

or the sixth power of the radius of the particles. There is therefore a very large increase of the intensity of the scattered light with the diameter of the particles. The intensity of the scattered light in various directions with respect to the direction of the primary beam is proportional to $(1 + \cos^2 \theta)$ where θ is the angle between the directions of the primary and scattered beams. The intensity of the scattered light is therefore greater in the forward and backward directions than sidewise and the radiation envelope is symmetrical about the 90° positions in forward and backward directions. Figure 23 shows the relative intensities of the scattered light in different directions, the radius vector to the envelope representing the intensity in that direction.

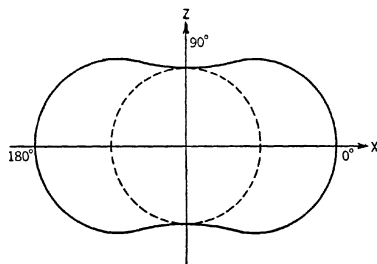


Fig. 23.—Radiation envelope for Rayleigh scattering.

In equation (17) the turbidity is directly proportional to the number of particles per cc., and hence, in principle, measurements of scattering may be used to enumerate particles and so to compute molecular weights. If in equation (17) the polarizability is expressed in terms of the refractive index, and n , the number of particles per cubic centimeter, in terms of the concentration, c , in grams per cc., the molecular weight, M , and Avogadro's number, N , the result is:¹²

$$H \frac{c}{\tau} = \frac{1}{M} \quad (18)$$

where:

$$H = \frac{32\pi^3}{3} \frac{\mu_0^2}{N\lambda^4} \left(\frac{\mu - \mu_0}{c} \right)^2 \quad (18a)$$

and μ and μ_0 are the refractive indices of solution and solvent, respectively. The dimensions of H are $\text{cm}^2 \text{ per g.}^2$. Applied to a dilute ideal solution of small particles in a solvent, the turbidity in equation (18) is the additional turbidity of the solute molecules over that of the solvent. In practice the turbidity of the solvent is subtracted from that of the solution to obtain the quantity to be used in the computation of molecular weights.

Equations (17) and (18) and their extensions which are discussed in the next section are based on the assumption that the particle is electrically isotropic, so that the direction of the induced electric moment is the same as that of the exciting field

of the electromagnetic radiation. In this case, the light scattered at right angles to the direction of an unpolarized primary beam is completely polarized with the direction of the electric vector perpendicular to the direction of the primary and of the scattered beam. In figure 23, the intensity of this component of the scattered light is indicated by the dotted circle, while the solid line indicates the total intensity, the difference between the two curves along any direction giving the intensity of the component vibrating parallel to the direction of the primary beam.

If the particle is not isotropic, the induced moment will tend to be set up in the direction of maximum polarizability, which on the average may not coincide with the direction of the exciting field. The light scattered at 90° from an unpolarized primary beam has then a component vibrating parallel to the direction of the primary beam, and is therefore not completely polarized. The *depolarization factor*, ρ , is the ratio of the intensities of the scattered light vibrating parallel to the direction of the incident light to that vibrating perpendicularly. In the scattering of an assembly of particles, any parallel component in the light scattered at 90° originating in particle anisotropy or in fluctuations in anisotropy from the mean represents a contribution additional to that depending on the mere number of particles. Since, according to equation (18) and its extensions yet to be discussed, other things being equal, the scattering increases with the molecular weight of the particle, molecular weights determined from light scattering at 90° will be too high if depolarization exists in the scattered light. The apparent molecular weight as obtained from measurements of the total scattering should then be multiplied by the Cabannes factor which is given by Debye⁵² as $6 - 7\rho/6 + 3\rho$. For high polymers such as polystyrene or cellulose acetate, ρ is not more than a few hundredths, and the correction amounts to 1–10%. A more detailed summary of the theory of depolarization is given by Doty and Kaufman.⁶¹

B. NONIDEAL SOLUTIONS OF SMALL PARTICLES

In practice, the determination of molecular weights by the scattering of light is limited to systems containing large molecules, which, however, even in dilute solution (of the order of 1%) show large deviations from ideality. It is therefore necessary to inquire how the scattering relations are influenced by such deviations. In this connection it appears to be most convenient, according to Debye, to consider the question in terms of Einstein's theory of scattering,⁵² in which scattering in a condensed system arises from inhomogeneities of density and also (in a solution) of concentration, which are spontaneously produced by the thermal motion of the molecules. In solutions of large molecules in normal solvents, by far the greater part of the scattering originates in the concentration fluctuations, the number of which will be proportional to the average thermal energy, kT , divided by an expression depending on the energy required to bring about a change in concentration. The latter quantity is related to

⁶¹ P. Doty and H. S. Kaufman, *J. Phys. Chem.*, **49**, 583 (1945).

the osmotic pressure, P , of the solution, and according to Debye equals $c \cdot dP/dc$, where c is the concentration. There will also be a term in the expression for the scattering depending on the efficiency of the fluctuations as scattering centers, which depends on the change of refractive index produced by a change in concentration, $\partial\mu/\partial c$. The scattering is then given by the equation:

$$\tau = \frac{32\pi^3}{3\lambda^4} \frac{kT}{c(\partial P/\partial c)} \mu_0^2 (c \cdot \partial\mu/\partial c)^2 \quad (19)$$

where μ_0 is the refractive index of the solvent.

For dilute solutions $(\mu - \mu_0)/c$ can be substituted for $\partial\mu/\partial c$, and, since for ideal solutions $P = cRT/M$ and $\partial P/\partial c = RT/M$, for such solutions of small particles equation (19) reduces to equation (18). Dilute solutions of large molecules deviate in practice from van't Hoff's law of osmotic pressures; a two-term expression for the concentration is necessary to express the osmotic pressure as a function of concentration, even at low concentrations:

$$P = (RT/M)c + Bc^2 \quad (20)$$

whence, substitution for $\partial P/\partial c = RT/M + 2Bc$ gives:

$$\tau = HcRT/(RT/M + 2Bc) \quad (21)$$

or:

$$\frac{Hc}{\tau} = \frac{1}{M} + \frac{2Bc}{RT} \quad (22)$$

where H is the same as in equation (18a).

Insofar as equation (22) is fulfilled, a plot of H multiplied by the reciprocal of the "specific turbidity" (turbidity per unit concentration) will give a straight line, whose intercept is the reciprocal of the molecular weight and whose slope is a measure of the interaction constant, B . This constant depends on the solvent, being greater for good than for poor polymer solvents.

Equation (22) is the fundamental basis of the determination of the molecular weights of large molecules in solution from measurements of the scattering of light, as long as no dimension exceeds about $1/15$ of the wave length. The measured quantities are μ_0 , the refractive index of the solvent, $(\mu - \mu_0)$, the difference in refractive index between the solution and the pure solvent, and the turbidity, τ , all measured for light of known wave length λ , in a series of solutions of known concentration, c , in grams per cubic centimeter. Equation (22) also applies to a solution of large molecules containing a distribution of molecular weights, provided that

the distribution is independent of concentration. The value of the molecular weight so obtained is the weight average molecular weight.⁵⁴

The different kinds of average for molecular weights of systems with a distribution of molecular weights, as furnished by various methods of measurement, are discussed by Lansing and Kraemer.⁶² The *number average*:

$$M_n = \frac{\sum n_i M_i}{\sum n_i} = \frac{\sum w_i}{\sum w_i / M_i}$$

is simply the total weight, $\sum w_i$, of the various species i divided by the total number of molecules, $\sum n_i$, where w_i , n_i , and M_i are, respectively, the weight, number of molecules, and molecular weight of the i th species. This is the quantity given by osmotic pressure measurements and other measurements depending on the colligative properties of solutions. The weight average is:

$$M_w = \frac{\sum n_i M_i^2}{\sum n_i M_i} = \frac{\sum w_i M_i}{\sum w_i}$$

Equation (18), $\tau = HcM$, shows that for any species the scattering depends both on the molecular weight and on the weight of the species per cc. It is the quantity $c_i M_i$ for the individual species which determines their contributions to the scattering. For the mixture:

$$\begin{aligned}\tau &= H(c_1 M_1 + c_2 M_2 + \dots) \\ &= H(c_1 + c_2 + \dots) \frac{(c_1 M_1 + c_2 M_2 + \dots)}{(c_1 + c_2 + \dots)} \\ &= HcM_w\end{aligned}$$

where c is the total concentration in grams per cubic centimeter, and M_w is the weight average molecular weight. The argument can be extended to the nonideal case.

It should, however, be noted that for solutions containing a distribution of molecular weights, the plot of Hc/τ may have an appreciable downward curvature. Reference 54 should be consulted for further information on solutions of mixed molecular weights.

For a polymer solution with a concentration of 0.01 g. per cc. in an organic solvent, $\mu - \mu_0$ is of the order 0.001, and H for wave length 5000 Å. is therefore about 1.5×10^{-6} . If the molecular weight of the scattering molecule is 100,000 the turbidity is therefore about 1.5×10^{-8} . Turbidities of this order can be conveniently measured by observations of the scattered light and, in contradistinction to the inverse variation of osmotic pressure with molecular weight, the turbidity increases with molecular weight. While, therefore, the osmotic method is most conveniently car-

⁶² W. D. Lansing and E. O. Kraemer, *J. Am. Chem. Soc.*, **57**, 1369 (1935).

ried out at the lower range of molecular weights of large molecules, say 20,000 to 200,000, the turbidity method is most convenient in the higher ranges.

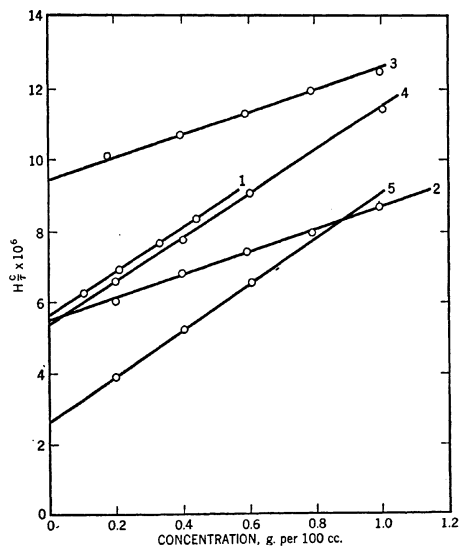


Fig. 24.—Plot of Hc/τ versus concentration for polystyrene fractions in methyl ethyl ketone.⁵⁴

TABLE III
COMPARISON OF MOLECULAR WEIGHTS OF POLYSTYRENE SAMPLES

Polystyrene	Molecular weight	
	Osmotic pressure	Scattering
No. 1	172,000	178,000
No. 2	198,000	182,000
No. 3	91,000	107,000
No. 4	—	190,000
No. 5	—	445,000

Examples of the linear relation between Hc/τ and c are given in figure 24, and in table III the molecular weights computed from the intercepts of the figure are compared with values determined from measurements of osmotic pressure.⁵⁴

The effect of mixed solvents on the turbidity of polymer solutions has

also been studied,⁶³ for example, mixtures of a good solvent and a precipitant. If (and only if) the refractive indices of solvent and precipitant are the same, the intercept of the plot of Hc/τ vs. c will give the correct molecular weight. The slope of the line decreases with increasing fraction of the precipitant, and the practical advantages of greater scattering at concentrations above zero and greater ease of extrapolation to zero concentration are achieved. If the refractive indices of the precipitant and solvent differ, both the slope and the intercept depend on the relative amounts of precipitant. In the systems studied by Ewart *et al.* it is shown by osmotic measurements that the varying intercepts do not indicate molecular weights depending on the amount of precipitant, and the reason is to be found in the preferential adsorption of the good solvent to the polymer. Scattering measurements therefore permit the study of such effects.

C. DILUTE SOLUTIONS OF LARGER PARTICLES

1. Dissymmetry of Scattering.—When a dimension of the scattering particle exceeds about $1/10$ to $1/15$ the wave length of the exciting light, Rayleigh's law of scattering ceases to hold. The variation of intensity with wave length is no longer as the inverse fourth power, the intensity of the scattered light is not symmetrical with respect to the forward and backward directions, being greater in the forward direction, and the angle for maximum polarization shifts to the backward direction. When the particle size becomes about half the wave length and is very uniform, pronounced maxima and minima appear in the angular distribution of the scattered light, the positions of which for a given monodisperse system depend on the wave length. If the suspension is illuminated with white light these maxima give rise to colored bands forming "higher order Tyndall spectra"⁶⁹ which in the hands of LaMer and his coworkers have proved to be a valuable tool in the study of the growth of spherical colloidal particles in monodisperse systems.^{69, 64}

These deviations from Rayleigh's law arise essentially from destructive interference of the light beams emitted from different parts of the scattering particle. This results in a general lowering of the intensity of the scattered light from the values predicted by Rayleigh's equations, and, since the difference in path length for light emitted from two volume elements of the particle is greater for light observed in the backward than in the forward direction, the greater probability of interference in the backward ray causes the amount of scattered light in the backward direction

⁶³ R. W. Ewart, C. P. Roc, P. Debye, and J. R. McCartney, *J. Chem. Phys.*, **14**, 687 (1946).

⁶⁴ I. Johnson and V. K. LaMer, *J. Am. Chem. Soc.*, **69**, 1184 (1947).

to be less than that in the forward direction. The radiation envelope of the scattered light is dissymmetric about the direction of 90° scattering.

Debye has recently simplified the problem of the theoretical determination of the radiation field surrounding large particles by assuming a negligible difference in refractive index between the particles and the medium and, in principle, obtained equations for the angular distribution of the scattered light for particles of any given shape.^{52, 55, 56} By suitable choice of solvents this condition can be fulfilled for organic polymers, and hence comparison of the angular distribution of scattering observed with the theoretical distribution for various models gives information on the size and shape of the particles.

For rodlike particles of length L , in comparison with which the thickness is small, the angular distribution of the intensity of the scattered light is given by:

$$I(x) = \frac{1}{x} \int_0^{2x} \frac{\sin x}{x} dx - \left(\frac{\sin x}{x} \right)^2 \quad (23)$$

where:

$$x = \frac{2\pi L}{\lambda} \sin \frac{\theta}{2}$$

θ being the angle between the scattered ray and the direction of the primary beam. In this expression the intensities are normalized to the value unity in the direction of the primary beam, and the factor $(1 + \cos^2 \theta)$ has been omitted since it is symmetrical about the direction of 90° scattering. Figure 25 shows the angular distribution of the intensity of the scattered light for various values of L/λ , for rodlike particles, as calculated by Oster, Doty, and Zimm.⁵⁶ For randomly kinked coils of roughly spheroidal shape, the mean square distance between the ends of which is R , the corresponding expression is:

$$I(v) = \frac{2}{v^2} [e^{-v} - (1 - v)] \quad (24)$$

where:

$$v = \frac{2}{3} \left(\frac{R}{\lambda} \right)^2 \left(2\pi \sin \frac{\theta}{2} \right)^2$$

For spheres of radius a the angular distribution is:

$$I(u) = \left[\frac{3}{u^3} (\sin u - u \cos u) \right]^2 \quad (25)$$

where:

$$u = \frac{4\pi a}{\lambda} \sin \frac{\theta}{2}$$

In practice, dissymmetry is usually measured by comparing the intensities I_f and I_b of the light scattered at two angles in the forward and backward directions, symmetrical to the 90° position, for example, 45° and 135° . If refraction occurs as the scattered beam leaves the cell, a correction must be made in setting the receiver at the appropriate angle in air. From equations (22) to (24) curves can be calculated for I_f/I_b as a function of the size parameters L/λ , R/λ , or a/λ for the particular forward and backward angles chosen for measurement, and, by comparing the experimental with the theoretical values, information on the size and shape of the

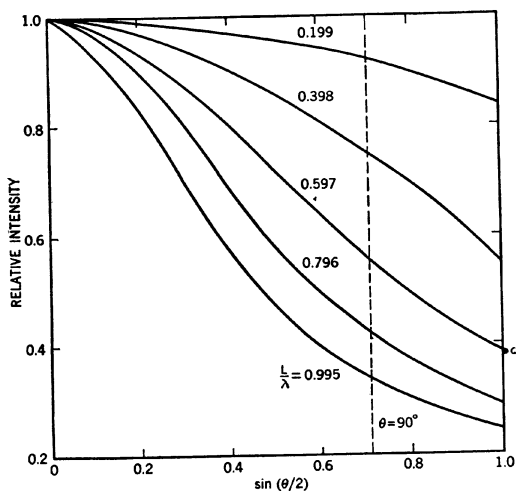


Fig. 25.—Relative intensity of scattering for rodlike particles.⁵⁸

particle can be derived. The wave length λ is the wave length in the solution, equal to the wave length in air divided by the refractive index of the solution. Figure 26 shows the curves for the *dissymmetry coefficient*, defined as $1 - I_f/I_b$, for coils and rods for angles of approximately 45° and 145° in the solution, as calculated by Doty, Affens, and Zimm.⁵⁸

In addition to interference effects between different parts of the same particle, interference can occur between the rays scattered by different particles, especially if there is any degree of regular orientation. In such cases the dissymmetry diminishes with concentration and it is the intrinsic dissymmetry, obtained by extrapolation to zero concentration, that is to be used in estimates of the particle size (see, for example, references 53 and 58).

2. Effect of Dissymmetry on Molecular-Weight Calculations.—As a consequence of interference effects in particles with a dimension greater than about $1/15$ the wave length of the exciting light, the turbidity of the solution observed in any direction other than the forward one is lower than it would be without interference, and the molecular weight computed from the observed turbidity is too low. A correction must therefore be applied which in effect gives the turbidity which would be observed if the interference effects did not exist. This correction can be computed for a specified shape from the angular distribution functions, equations (22), (23), and (24).^{55, 56} For example, if the particle is assumed to be rodlike, figure 25 shows that the scattering at 90° for a system of rods whose length is 0.4 times the wave length is 0.74 as intense as it would be in the absence of destructive interference. The ordinates corresponding to 90° scattering are shown by - - - cutting the abscissae axis at $\sin(\theta/2) = \sin 45^\circ = 0.707$. The corrected scattering to be used in molecular-weight determinations is thus $1/0.74 = 1.35$ times the observed. It is convenient to construct a curve of turbidity corrections *vs.* dissymmetry for various assumed particle shapes. The dissymmetry for any value of the size parameter and particle shape is calculated by taking the ratio of the intensities at the appropriate values of $\sin \theta/2$ for the forward and backward angles as plotted in curves like those in figure 25. Figure 26 shows curves of the dissymmetry coefficient, as a function of the size parameter for rods and kinked coils, as given by Doty, Affens, and Zimm.⁵⁸ The forward and backward angles are approximately 45° and 135° , the actual angles, in this special case, differing somewhat with the solvent.

With the aid of curves such as those in figures 25 and 26, we can proceed to construct curves of turbidity corrections against dissymmetry. Returning to our example of rodlike particles of length equal to 0.4 times the wave length, we find that the corresponding dissymmetry coefficient is 0.4, and the turbidity correction is 1.35. Proceeding thus, we can construct curves of turbidity corrections against dissymmetry coefficients for various particle shapes. Figure 27 shows such a curve for rodlike particles.

As an example of the results achieved by measurements of scattering and dissymmetry, the observations of Oster, Doty, and Zimm⁵⁶ on tobacco mosaic virus may be quoted. The molecular weight, determined from turbidity measurements corrected for dissymmetry, was 40×10^6 , the length of the particles (assumed rodlike) from dissymmetry measurements was $270 \pm 15 \text{ m}\mu$, while electron microscope observations gave a length of $262 \text{ m}\mu$, and measurements of viscosity $260 \text{ m}\mu$. The satisfactory agreement of the value estimated from scattering experiments with those obtained from other observations is strong support of the soundness of the scattering method.

To recapitulate the general procedure for determining molecular weights by measurements of turbidity, the scattering of monochromatic light is measured as a function of concentration at 90° to the incident direction, and also at two angles in the forward and backward directions, symmetrical with respect to 90° , say 45° and 135° . If in the latter two measurements the readings are the same, the particles are small compared with the wave length of the measuring light, and the extrapolation of the curve of Hc/τ vs. c to zero concentration will give the weight average molecular weight.

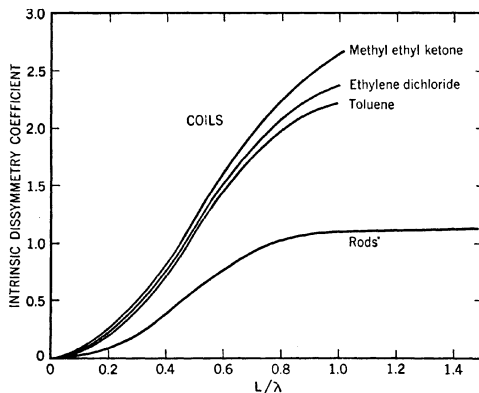


Fig. 26.—Dissymmetry coefficients as a function of size parameter for rods and coils.⁴⁸

If the light scattered at 90° for unpolarized incident light is not completely polarized with the electric vector vibrating perpendicular to the direction of the incident beam, a correction for orientation scattering is necessary, reducing the molecular weight. In practice, this correction is usually small.

If the intensities of scattering in two directions, forward and backward, symmetrical to the 90° direction are not equal, the particle size is greater than about one-tenth of the wave length. The dissymmetry is measured as a function of concentration, and the intrinsic dissymmetry, depending wholly on intramolecular interference, is found by extrapolation to zero concentration. If the shape of the particle is known, the dissymmetry coefficient will permit an estimate of the size parameter, L/λ . In principle, the shape of the particle can be determined from a detailed knowledge of the angular distribution of the intensity of scattering; calculations are available for this relation for spherical, rodlike, and open-coil particles. When the shape has been decided upon, the correction factor to be applied

to the measured turbidity to use in molecular-weight calculations is read off from a curve showing the correction as a function of the dissymmetry. The molecular weight is then computed from the intercept of the plot of H times the reciprocal of the corrected specific turbidity vs. concentration.

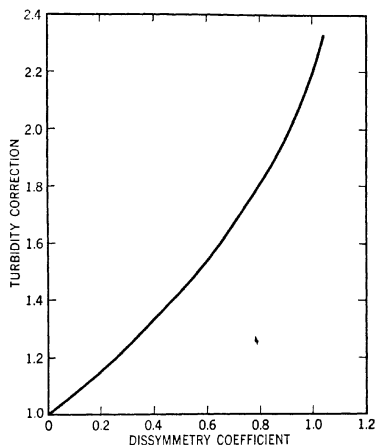


Fig. 27.—Turbidity correction as a function of dissymmetry coefficient for rodlike particles.⁵⁵

D. VARIATION OF WAVE-LENGTH EXPONENT WITH PARTICLE SIZE

In addition to the effects just discussed on intensity and angular distribution of intensity, the presence of particles of size greater than about one-tenth the wave length causes deviations from Rayleigh's law in the wave-length dependence and on the polarization relations, which may be used to secure information on the size of particles.^{59, 65} The intensity of scattering, which varies inversely as the fourth power of the wave length for particles small in comparison with the wave length, varies inversely as smaller powers as the particle size increases. The turbidity, τ , in equation (16) can then be expressed as a function of wave length in the relation:

$$\tau = k\lambda^{-n} \quad (26)$$

where k is independent of wave length if the scattering particles have the same optical dispersion as the medium, and n depends on the particle size.⁶⁶ A plot of $-\log \tau$ (or of the logarithm of the optical density of the turbid

⁵⁵ W. Heller, H. B. Klevens, and H. O. Oppenheimer, *J. Chem. Phys.*, **14**, 566 (1946).

⁶⁶ W. Heller and E. Vassej, *J. Chem. Phys.*, **14**, 565 (1946).

medium in a transmission measurement) against $\log \lambda$ will then give a straight line whose slope is n , 4 for Rayleigh scattering and a smaller number for larger particles. Figure 28, according to Heller, Klevens, and Oppenheimer⁶⁵ shows the variation of n with particle size for aqueous emulsions of polystyrene containing spherical particles with a ratio of refractive index to that of the medium of 1.23 to 1.25. Each experimental point represents a substantially monodisperse system of size known from electron microscope observations. The data may be applied to particle size determinations in any type of disperse system containing nonabsorbing spherical particles of refractive ratio 1.24 ± 0.01 . The variation of n

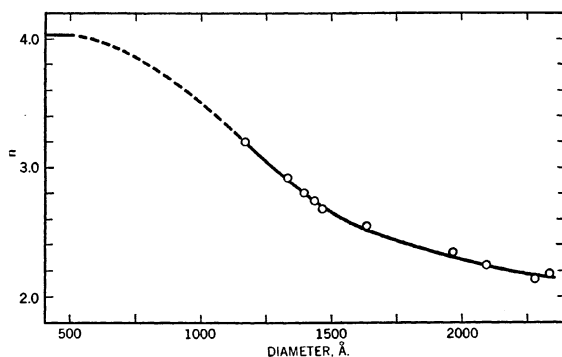


Fig. 28.—Variation of wave-length exponent with particle diameter for dielectric spheres.⁶⁵

with λ has been used to detect a small amount of true absorption in a scattering medium.^{66, 67} For a purely scattering process the exponent n of the apparent "absorption coefficient" (turbidity) cannot exceed the value 4, while for true absorption, n may be much greater.

3. Experimental Methods for Molecular-Weight Determination by Light Scattering

The determination of molecular weights of large molecules by light scattering requires: (1) some measure of the intensity of the scattered light, (2) some measure of the dissymmetry of intensity in forward and backward directions, and (3) a measure of the refractive-index difference between solvent and solution, all as a function of concentration and at known wave lengths. The refractive index of the pure solvent must also

⁶⁷ W. Heller and E. Vassey, *Phys. Rev.*, **63**, 65 (1943).

be known, and it may be desirable to know the state of polarization of the scattered radiation. The methods of measurement of refractive indices and of differences in refractive index are described in Chapter XX.

A. LIGHT SOURCES

Light of known wave length is most conveniently procured by filter isolation of a line in a line-spectral source. Mercury arcs of the AH-4 type are frequently used (Chapter XXI, Section 2A). The green line, of wave length 5461 Å., as isolated by the "mercury achromatic" filter combinations supplied by the various manufacturers of filters (this chapter, page 1421) is convenient for colorless solutions. Fluorescent solutions must be examined with light of wave length which they do not absorb enough to excite fluorescence, and the solvent must obviously be nonfluorescent. For visible illumination the purified solvents normally used for high polymers are satisfactory in this respect, and common-sense precautions must be taken to avoid the introduction of fluorescent impurity, for example, from corks, rubber tubing, or rubber stoppers. The mercury spectrum gives a number of strong, suitably placed lines throughout the visible, except in the red (Chapter XXI, page 1281); red illumination would normally be avoided because of the relatively poor scattering of this light, but when necessary a cadmium-mercury arc could be employed.

Debye⁶⁸ has described a simple arrangement which secures adequate constancy of the AH-4 mercury lamp. The current from the mains (a. c.) supplies the primary of a voltage-regulating autotransformer of adequate power, the output of which is passed through a ballast lamp (General Electric B47) in series with the primary of a step-up transformer. The function of this transformer is to compensate for the voltage drop across the ballast lamp, the secondary delivering the rated input voltage to the primary of the lamp transformer.

B. MEASUREMENT OF TURBIDITY

1. **Transmission Method.**—Equation (16) shows that, under appropriate conditions of measurement, the density of a turbid solution of thickness x showing only conservative absorption is $D = \tau x/2.303$ or $\tau = 2.303D/x$. If τ is 0.005, the density of a 20-cm. column is about 0.04, corresponding to a transmission of 91%. High precision in the measurement of small densities is therefore necessary in the transmission method, which is limited to solutions of relatively high turbidity. The device of adding an appropriate precipitant to the solvent⁶³ to increase turbidity should be useful in this method.

⁶⁸ P. P. Debye, *J. Applied Phys.*, **17**, 392 (1946).

The Beckman spectrophotometer (Chapter XXI, page 1324) has been *used by Heller and coworkers⁶⁵⁻⁶⁷ to determine particle size from determinations of the wave-length exponent of scattering (this chapter, page 1461).* Doty, Affens, and Zimm⁵⁸ have used this instrument to measure the absolute turbidity of a low molecular weight polymer (without dissymmetry) to serve as a calibration standard. By measuring the transmission as a function of wave length and plotting the density against the logarithm of the wave length, a straight line was obtained, from which the turbidity at a specified wave length in the range could be read with greater precision than from a single direct measurement. Photoelectric filter photometers could be used for this type of measurement. The apparatus of Debye illustrated in figure 30 can be used for transmission methods, and a photoelectric transmission turbidimeter which might prove useful for small turbidities is illustrated in figures 37, 38, and 39 (page 1484).

A precaution to be taken in transmission measurements of turbidity is to keep the aperture of the receiver small enough to exclude the entrance of laterally scattered light into the light-sensitive unit; if this is not done the measured density will be low.

2. Measurement of Lateral Scattering.—This method, in which the light scattered at 90° or at some other known angle is measured, necessitates the estimation of a low intensity with a precision of a few per cent. The measurement is similar to that of fluorescence, with the difficulty that, since the exciting and scattered radiation have the same wave length, the use of complimentary filters to exclude scattered and reflected incident light from the measured beam is excluded. Stringent avoidance of reflected incident light from parts of the apparatus in the direction of the photometer receiver by the use of baffles and light traps is therefore necessary (Chapter XXI, page 1375).

Visual and photoelectric methods are currently used. Both have advantages: visual methods employing photometers such as the Pulfrich instrument (this chapter, Section V) are capable of high precision, have the advantage of a low aperture for the photometer, and are relatively simple to maintain, but have the disadvantages associated with subjective measurements; photoelectric methods usually involve the maintenance of a sensitive amplifier. Normally, however, good commercial amplifiers of high sensitivity give little trouble in maintenance.

In visual methods of turbidimetry, the precautions pertinent to any type of visual photometry should be taken. Measurements should be made in a darkened room with adequate illumination of scales and manipulative parts, shielded to exclude glare from the observer. The observer's eyes should be adapted to the dark before beginning readings, and the observer should be in a position free from strain or discomfort. Usually two adjacent photometric fields must be matched in brightness,

and often the most accurate procedure is to set so that first the one and then the other field is just perceptibly darker than its neighbor and to average the corresponding readings. A number of quick readings made one after the other will, in general, yield greater accuracy when averaged than a few made with prolonged effort at matching, which often causes the field to swim before the eyes.

Visual Turbidimeter.—A visual instrument, employing a Pulfrich photometer in conjunction with a suitable chamber holding the source and scat-

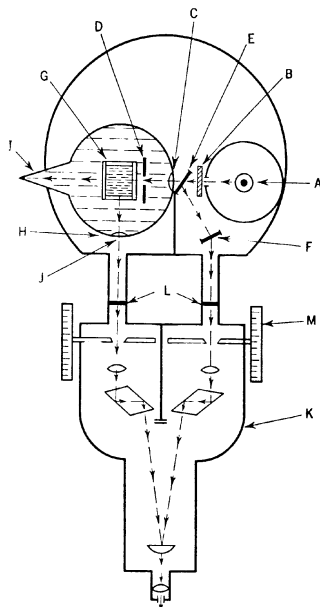


Fig. 29.—Instrument for the visual measurement of intensity scattered at 90° , according to P. M. Doty.⁵⁵

tering cell, is described by Stein and Doty.⁵⁵ In figure 29, light from a vertical mercury arc (100-watt AH-4), made monochromatic at 5461 \AA . by filters *B* and collimated by lens *C* and square diaphragm *D*, illuminates the scattering solution contained in a square cell, *G*. *G* is set in a vessel, *H*, through which water from a thermostat is circulated for temperature control. Light transmitted by the solution is received by the conical light trap, *I*. Some of the light scattered at 90° is collected by lens *J* and projected at one of the slits of a Pulfrich photometer, *K*. The intensity of the scattered light is measured with respect to a comparison beam isolated

from the incident beam by reflection at the glass plate, E , which throws a small constant fraction of the incident light on an opalescent glass plate, F . Neutral filters may be introduced at L in order to limit the intensity in calibrating the instrument. The scattered and reference beams are compared in the usual way, the ratio of intensities being read from the scales on the dials adjusting the slit areas of the photometer.

Absolute Turbidity.—In order to obtain the absolute turbidities necessary for molecular-weight determinations, the readings for instruments employing the principle of 90° scattering must be calibrated in terms of the turbidities of absolute standards. One method of absolute determination of a low molecular weight polymer in a suitable solvent, for example, polystyrene in butanone, by means of the Beckman spectrophotometer or similar instrument, has already been described.

In another method, the intensity of the scattered light is compared with that of a small known fraction of the incident light diverted laterally. One procedure is to measure the diffusely reflected light, suitably weakened by neutral filters, of known transmission, from a prism of magnesium carbonate mounted with the reflecting surface at 45° to the incident beam. In the visual turbidimeter of Doty and Stein, this block, mounted in a glass cell, is substituted for the scattering solution. If I_s is the intensity scattered at 90° per unit area of the photometric field, at a distance r from the solution, and I_i is the corresponding intensity reflected from the magnesium carbonate, it can be shown that:⁵⁵

$$\frac{I_s}{I_i} = \frac{i_0 h \pi r^2}{IR \sin 45} \quad (27)$$

where i_0 is the scattered intensity at distance r per unit volume of the solution, h the depth of the illuminated solution in the line of view from the photometer, and R the reflectivity of the magnesium carbonate surface (about 0.97). This ratio is given by the ratio of the respective dial readings for solution and magnesium carbonate at photometric balance with the comparison beam on the right side of the photometer. The total amount of scattered light emitted per unit volume of the solution is found by integrating over a sphere, taking account of the angular distribution of Rayleigh scattering, whence:

$$\tau = \frac{16\pi i_0 r^2}{I} \quad (28)$$

Substituting for $i_0 r^2/I$ from equation (27):

$$\tau = \frac{16\pi R \sin 45 I_s}{3h I_i} \quad (29)$$

It will be noticed that distance r is eliminated from the final equation.

Debye⁵² determines the turbidity of polystyrene solutions in toluene to be used as standards by a special apparatus, using photographic photometry. A parallel monochromatic beam is passed through a small rectangular cell containing the polymer solution, and the light scattered at 90° , limited by a small aperture, falls on a photographic plate placed a few inches from the cell. After passing through the scattering cell, the primary light enters a second cubical cell containing a hollow cube immersed in water. Three vertical sides of the cube are glass, the fourth is open. The light enters at the open side, and the cube is so placed that the beam undergoes three reflections at 45° , finally leaving the cube through the open side, and passing from the cell in a direction perpendicular to the primary beam to fall on the photographic plate near the scattered beam. After the three reflections, the intensity, which can be calculated by Fresnel's law⁶⁹ from a knowledge of the refractive indices of glass and water, is comparable to the scattered intensity. The densities of the two photographic images are measured by means of a microphotometer. On the same plate exposures are made through a screen transmitting a graduated series of intensities in a known ratio ("step-tablet") so that the density-intensity relation for the plate can be found. From this relation the relative intensities of the scattered and reflected beams can be found, and the turbidity of the solution obtained in a similar way to that already described for the reflection from magnesium carbonate. The normal precautions for photographic photometry should be taken (pages 1312 and 1381).

Photoelectric Turbidimeters.—Electron-emissive cells with electronic amplification are used in photoelectric turbidimeters for low turbidities.

⁶⁹ The reflected fraction of the light intensity incident normally on the surface between two transparent media, the ratio of whose refractive indices is μ , is $\left(\frac{\mu - 1}{\mu + 1}\right)^2$. For other angles of incidence the amounts reflected depend on the state of polarization of the incident light. In the general case, if i and r are the angles of incidence and refraction, measured from the normal to the surface, the reflected fraction of the incident intensity in which the electric vector vibrates perpendicularly to the plane of incidence is

$$R_{\perp} = \frac{\sin^2(i - r)}{\sin^2(i + r)}$$

and the fraction reflected of light vibrating parallel to the plane of incidence is:

$$R_{\parallel} = \frac{\tan^2(i - r)}{\tan^2(i + r)}$$

If the incident light is ordinary unpolarized light, the fraction reflected, which may be regarded as compounded of two equal intensities polarized at right angles to each other, is $(R_{\perp}/2) + (R_{\parallel}/2)$.

Linear response to light intensity is desirable, and, therefore, vacuum cells in conjunction with linear amplifiers should be used. Some circuits are mentioned in Chapter XXI, page 1317; various commercial amplifiers are available.⁷⁰

Figure 30 sketches the optical arrangements in a versatile instrument described by Debye⁶⁸ for the measurement of scattering at 90° , of transmission, and of the angular distribution of scattering. Three interchangeable sources are used, mounted in separate housings: a high-intensity tungsten-filament lamp, a high-pressure, water-cooled mercury arc, H-6 (Chapter XXI, page 1285), and, for most operations, a mercury arc of the H-4 type, stabilized by a ballast lamp circuit (this chapter, page 1463). Light from the source illuminates the opal glass, *O*, in front of

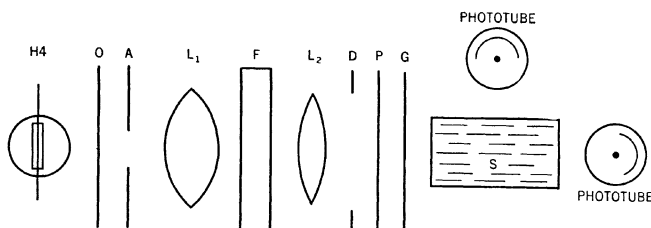


Fig. 30.—Optical design of photoelectric turbidimeter, according to P. P. Debye.⁶⁸

a slide, *A*, containing various interchangeable apertures, passes through the monochromatic filter, *F*, and is rendered parallel by the lens, *L*₂. The iris diaphragm, *D*, limits the beam to a size suitable for passage through scattering cell *S*; a Polaroid *P* or a plain glass slide, *G*, can be introduced, if desired, into the beam. The glass slide is used in calibrating the instrument for transmission measurements; a calculable small fraction of the light is reflected according to Fresnel's law and the corresponding change in the meter reading is noted. Lens *L*₁, of greater aperture ratio than *L*₂, is removable, and used to focus aperture *A* at the center of the scattering cell, *S*, when higher intensities are required.

The phototubes (RCA 929) can be connected interchangeably to a d.-c. amplifier, made by the *Photovolt Corporation*. The intensities are read on a taut-suspension galvanometer, shunted so as to possess a variable sensitivity range. The constancy of the source and amplifier system is checked before and after a series of measurements by reflecting the primary beam through a dark glass filter from a magnesium carbonate block at 45° on to a photocell. This arrangement is not shown in the diagram. Other photo-

⁷⁰ For example, from *Photovolt Corporation*, 95 Madison Avenue, New York, N. Y.

tubes are mounted as shown near the scattering cell for the measurement of 90° scattering and of transmission. The latter tube is mounted on an arm movable about a perpendicular axis passing through the bottom of the apparatus so that it can be rotated into various positions with respect to the primary beam, denoted by an indicator outside the case which encloses the whole apparatus; angular distribution of scattering at angles up to 125° with respect to the primary beam can be studied. In measurements of angular distribution, either a cylindrical cell must be used, or, if the cell is rectangular, corrections must be made for varying reflection and absorption

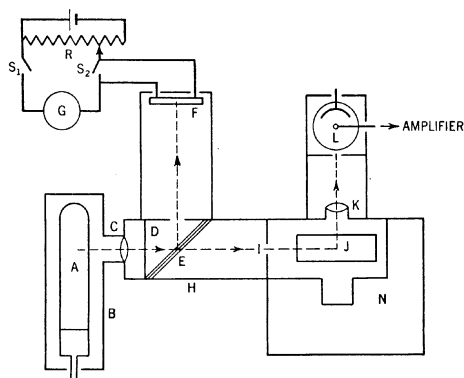


Fig. 31.—Photoelectric turbidimeter of Doty, Wagner, and Singer.⁵³

and for different volumes of the scattering liquid in the field of view of the photocell at different positions. These corrections can be eliminated by mounting a small mirror within the solution at 45° to the horizontal so that it may be moved through a semicircular path. Underneath the flat bottom of the scattering cell is mounted a phototube, which moves on the same semicircle as the mirror. This cell therefore receives light from a constant fixed volume of the scattering solution as emitted at various angles with respect to the incident beam, and the correction for reflection at the bottom of the scattering cell is the same at all positions. This whole apparatus with the exception of the amplifier and galvanometer is mounted in a rigid metal case in such a way that the various movable parts can be set in precisely reproducible positions.

An example of a simpler instrument is the photoelectric turbidimeter used by Doty, Wagner, and Singer⁵³ for measuring the dependence of polymerization on temperature (Fig. 31). A parallel beam from mercury

lamp *A* (100-watt AH-4), in housing *B*, leaves lens *C* and monochromatic filter, *D* (5461 Å.), mounted in the blackened wooden box, *H*, and passes through an aperture, *I*, so that a beam about 0.5 inch in diameter enters the scattering solution in vessel *J*. The beam is stopped down so that no light hits the sides of the cell. A portion of the light scattered at 90° is collected by lens *K*, mounted on the outside of the thermostat, and focused on the vacuum phototube, *L*. The output is amplified and measured by a sensitive galvanometer.⁷¹ The sensitivity of the instrument is about 2×10^{-6} foot-candles per scale division for blue light; greater sensitivity can be secured by switching out a shunt across the galvanometer. Turbidities as low as 3×10^{-5} can be measured. The cell, lens, and phototube are so placed that only light from the center of the solution reaches the phototube. Enough liquid is placed in the cell to avoid reflections from the meniscus. The scattering cell is mounted in a metal housing which fits into a thermostated water bath, *N*.

To compensate for variations in the intensity of the primary beam, the scattered radiation is measured relatively to a small fixed fraction of the primary beam, which is read at the same time. Two pieces of plate glass, *E*, mounted at 45° to the incident beam reflect a portion of the light on to a Photronic cell, *F*. To minimize temperature changes, to which the output of this cell is somewhat sensitive, it is placed about 6 inches away from the rest of the apparatus. The current developed by the Photronic cell is measured by a "zero potential" circuit (this chapter, page 1416). Potentiometer *R* is adjusted until microammeter *G* records the same reading (a) with switch *S*₁ closed and *S*₂ open, and (b) with both switches closed. To check the linearity of the response of the Photronic cell to intensity, the transmission of an optically flat piece of glass is measured. Different transmissions are obtained by stacking several pieces of the glass together, the transmission of a pile of *n* plates being taken as T^n where *T* is the transmission of a single plate. The linearity of the phototube-amplifier circuit used for measurement of the scattered light is verified in the same manner.

Before every set of measurements the instrument is calibrated by means of a known standard of 1% polystyrene in tributylacetyl citrate. The readings are taken by first adjusting the galvanometer to zero with a shutter in the light beam, opening the shutter, noting the deflection, closing the shutter, and taking as the zero the mean of the two readings with the shutter closed. Three such sets of readings are taken for each measurement of scattering.

Let p_0 and c_0 be, respectively, the deflections of the galvanometer indi-

⁷¹ The entire photometer unit was made by Photovolt Corporation, 95 Madison Ave., New York, N. Y.

cating the intensity of scattered light, and the meter connected to the Photronic cell when the standard, of absolute turbidity K , is in the measuring cell, and let p_s and c_s be the corresponding readings for the unknown solution. Then the turbidity of the solution is:

$$\tau = \frac{K p_s c_0}{p_0 c_s} \text{ cm.}^{-1}$$

The electron multiplier seems adapted to the measurement of scattered light. Electron multiplier photometers with power supply and galvanometer are commercially available.*

C. MEASUREMENT OF DISSYMMETRY

Debye has used photographic photometry in studies of the angular distribution of the intensity of scattered light.⁵² In one apparatus, the light scattered by a parallel beam leaves the solution through a flat glass plate in the side of the cell, passing through a small circular hole in a metal plate near the glass plate. The scattered light is received on a photographic film bent to a semicircle around the hole. The density of the photographic image is measured as a function of the angle of scattering by means of a microdensitometer, and calibration marks are placed on the film for the determination of the density-intensity relationship. Corrections must be made for refraction at the cell walls and for the different elements of volume from which the scattering observed at different angles originates. The primary beam in this apparatus is absorbed in a light trap. By the use of an octagonal cell, scattering at 45° , 90° , and 235° can be measured. The appropriate faces of the cell are provided with screens with a small circular hole for the passage of the scattered light, followed by a tube acting as collimator. No corrections for refraction are required.

Provided that a microphotometer is available, the photographic method has the merit of instrumental simplicity but is subject to the pitfalls besetting photographic photometry. With care in calibration and processing the method should be accurate within a few per cent but most investigators would probably prefer photoelectric adaptations of these methods.

Stein and Doty⁵⁵ describe an apparatus which can be used in conjunction with a Pulfrich photometer for the measurement of beams scattered at two angles symmetrical to the 90° position (Fig. 32). The source is a mercury lamp mounted in a water-cooled housing, *A*. The narrow slit, *B*, in the housing is placed at the focus of convex lens *C*, from which a narrow rectangular pencil of parallel light, rendered monochromatic by filters, *D* and

* *Farrand Optical Co.*, Bronx Boulevard and East 238 Street, New York, N. Y.

collimated by two vertical slits, *E* and *F*, $\frac{1}{8}$ inch wide, passes through the solution in cell *G*, which is 100 mm. long, 20 mm. wide, and 50 mm. high. The cell is held by metal guides in the center of the blackened metal box, *H*. After leaving the cell, the exciting light passes through the exit hole in *H*.

Two mirrors coated on the front surface, *J* and *K*, are mounted so as to reflect two scattered rays symmetrical with respect to the 90° axis out of the box through two circular holes in the front wall. A central barrier, *L*, prevents the access of stray light to the mirrors. The mirrors are mounted on bases with dowel pins fitting into holes through the bottom of the box.

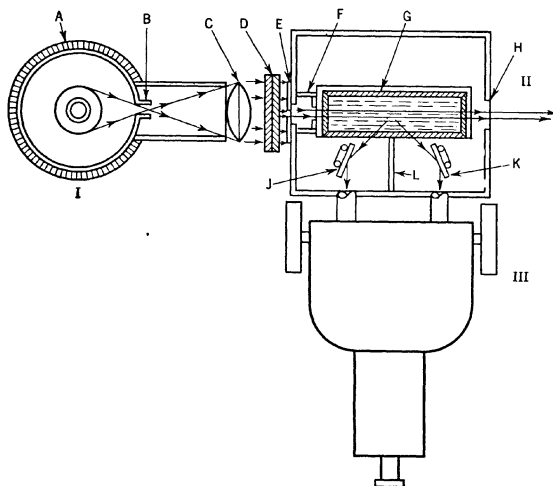


Fig. 32.—Instrument for the measurement of dissymmetry of scattered light, according to P. M. Doty.⁶⁶

Several sets of holes are provided so as to permit variation of the angles symmetrically about the 90° axis. After leaving the box, the two scattered light beams enter the apertures of a Pulfrich photometer and are compared in the usual way (page 1429). At photometric balance, the ratio of intensities of the two beams is inversely proportional to the respective dial readings.

The symmetry of construction of the instrument can be checked by placing a solution of fluorescein in the cell and observing whether the intensity of the two fluorescent beams received by the mirrors is the same. The presence of unsymmetric stray light in the photometric beams can be detected by measuring the two fluorescent beams at a number of concentra-

tions. A dissymmetry increasing with decreasing concentration of the fluorescein indicates stray light.

Besides its use in studying the relative intensities of two beams scattered symmetrically in the forward and backward directions, this instrument can be used to study the angular distribution of the scattered light. One of the mirrors is removed and the position of the other varied; the ratio of intensities at a selected angle to that at 90° can then be determined.

In comparing intensities with this instrument at angles not symmetrical about the 90° axis, several corrections must be made. These are illustrative of the type of correction that frequently arises in studies of angular distribution, and are quoted here in the hope that they may be of assistance in other cases than with this particular instrument.

(a) At different angles the receiver "sees" different volumes of scattering solution. This can be corrected approximately by multiplying the measured intensity by $\sin \theta$.

(b) While in measurements at angles symmetrical to the 90° axis, both mirrors receive their light from the same volume of scattering solution, situated equidistant from the points of exit of the two photometric beams from the scattering cell, this is not true in measurements in which one of the mirrors is removed, and the scattered beam reflected by the other mirror is compared with the beam scattered at 90° . If the right-hand mirror is removed, the volume of solution from which the 90° beam comes is in front of that giving the other beam and a correction must be made for the attenuation of the primary beam in passing between the two sites. The correction can be found by removing both mirrors and comparing the two 90° beams then observed by the photometer. The correction for the conservative absorption is approximately half the ratio of the two intensities thus observed.

(c) Losses by reflection at the solution-glass-air interfaces are a function of the angle. This correction is:

$$\frac{(1 - r_1^2)(1 - r_2^2)}{1 - r_1^2 r_2^2} + \frac{(1 - r_1'^2)(1 - r_2'^2) \cos^2 \theta}{1 - r_1'^2 r_2'^2} \\ \hline 1 + \cos^2 \theta$$

where:

$$r = \frac{\sin(\phi_1 - \phi_2)}{\sin(\phi_1 + \phi_2)} \text{ and } r' = \frac{\tan(\phi_1 - \phi_2)}{\tan(\phi_1 + \phi_2)}$$

The subscripts 1 and 2 on the r 's refer to the liquid-glass and glass-air interfaces; ϕ_1 and ϕ_2 are the angles of incidence and refraction.

As an example of the determination of molecular weight and molecular size, we quote data in table IV from Oster, Doty, and Zimm⁵⁶ on tobacco mosaic virus in aqueous solution.

TABLE IV
DATA ON TOBACCO MOSAIC VIRUS

Concn., g./cc., $\times 10^4$	$\tau \times 10^3$, cm. $^{-1}$	I_f/I_b	Turbidity correction factor	$\tau_{\text{corr.}} \times 10^3$, cm. $^{-1}$	$(Hc/\tau_{\text{corr.}}) \times 10^3$
2.9	2.26	1.67	1.59	3.60	2.54
1.4	1.035	1.78	1.74	1.80	2.54
0.97	0.68	1.83	1.81	1.23	2.49

The value of H was 3.15×10^{-6} . Column 2 of the table gives the observed turbidity measured at 90° for wave length 5461 \AA . against a standard whose turbidity had been determined by transmission measurements. Column 3 contains the ratio of forward to backward scattering at angles in air of 42.5° and 137.5° from the direction of the incident beam. The particles are assumed to be rod shaped, and the correction factors corresponding to the dissymmetries of column 3 are read off from figure 27 and entered in column 4. The "corrected" turbidity, which would have been observed at 90° in the absence of the factors causing dissymmetry, is contained in column 5, and the quantity $Hc/\tau_{\text{corr.}}$ in column 6. The value of $Hc/\tau_{\text{corr.}}$ at zero concentration is about 2.5×10^{-8} , the reciprocal of which, 40×10^6 , is the molecular weight.

To obtain information about the particle size, the dissymmetry at zero concentration is estimated by extrapolation of the observed dissymmetry-concentration relation in column 3. The extrapolated value for the "intrinsic dissymmetry" is 1.94. From figure 26, this corresponds to L/λ equal to 0.66. The wave length of the exciting light is $546 \text{ m}\mu$ in air, and therefore in the solution, whose refractive index is practically that of water, 1.34, the wave length is $546/1.34 = 409 \text{ m}\mu$. The length of the particles, assumed to be rigid rods, is therefore $0.66 \times 409 \text{ m}\mu = 270 \text{ m}\mu$. As has already been mentioned, this value agrees with that obtained from electron microscopic and viscosity studies.

D. MEASUREMENT OF DEPOLARIZATION

Of the various possible methods of measuring the state of polarization of scattered light, only the visual method used by Doty and Kaufman⁶¹ will be described. In Chapter XXI (page 1379), reference is made to some objective methods, photographic and photoelectric, used in the study of the polarization of Raman spectra, which could be adapted to the study of the unmodified scattering.

Figure 33 shows the apparatus. From the source, an AH-4 mercury lamp in a water-cooled housing, a beam is made parallel by lens L , of 18-cm. focal length, and rendered monochromatic for 5461 \AA . by a Corning

glass combination, *F*. The light passes through the cell, *C*, $3 \times 3 \times 10$ cm. high, as a beam defined by the iris diaphragm, *I*, so as not to touch the side walls. The cell is contained in a housing, *H*, painted dull black inside, furnished with an exit port for the primary beam after it leaves the cell, and with a side port for the exit of the scattered light at an angle of 90° to the primary beam. The cell is introduced into *H* by a square opening which secures reproducibility of position. If polarized primary light is desired, a Polaroid *P* is introduced into the beam, which can be rotated to give vertically or horizontally vibrating light. This is removed for studies of the scattering of an unpolarized primary beam.

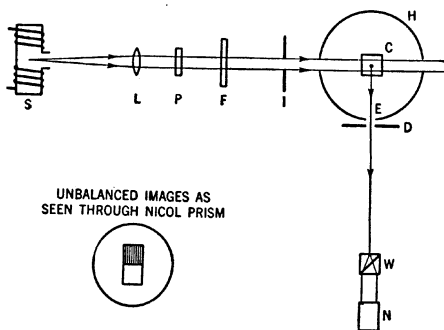


Fig. 33.—Measurement of depolarization⁷¹ by Cornu method.

The principal axes of a Polaroid can be found by looking through it at the image of a frosted bulb reflected at an angle of $50\text{--}60^\circ$ from the normal by a glass plate, remembering that the reflected light is partially polarized with the electric vector vibrating horizontally. When the Polaroid is rotated about its center until the image appears darkest, it is then absorbing horizontally vibrating light and transmitting vertically vibrating light.

Another rule useful for determining the direction of vibration of polarized light is that the polarized light transmitted by a normally cut nicol vibrates parallel to the shorter diagonal of the end surface of the prism.⁷²

In front of the exit port, *E*, for the scattered light is a diaphragm, *D*, with an opening 0.5 cm. square. The beam defined by this aperture enters a Wollaston prism, *W* (Chapter XXIII, Section V, 2A), which separates the horizontal and vertical components. The Wollaston is placed at such a distance from *D* and so oriented about its axis normal to the faces that the two images of the diaphragm, *D*, are one above the other and in contact (Fig. 33). One image, produced by vertically vibrating light, will be

⁷² R. W. Wood, *Physical Optics*. 3rd ed., Macmillan, New York, 1934, p. 377.

brighter than the other, which will be absent if the depolarization is zero. The two images are examined through a nicol prism, N , mounted on a divided circle read to 0.1° , on the same axis as W , and rotatable through 360° . The nicol is set so as to extinguish the brighter image. Rotation of the nicol a few degrees on either side of the extinction point will cause the two images to appear equally bright, if the depolarization is small. The angle between match points is read as 2θ . For increased accuracy, the balance positions are determined as the average of settings at both sides of the true equibrightness points. There is another set of match points about an extinction point 180° from the first. The depolarization is given by $\tan^2 \theta$.⁷³ In general, the angle in Doty and Kaufman's experiments could be measured with an accuracy of $\pm 0.05^\circ$. Calibration with liquids of known depolarization was satisfactory.

As in all depolarization measurements careful check of the apparatus against systems of known polarization should be carried out, to avoid polarizations introduced by the instrument. Table V contains values of the depolarization factors for the light scattered from an unpolarized primary beam by pure liquids, suitable for calibration, and a few examples of the depolarization values for polymers found by Doty and Kaufman. Their article should be consulted for a discussion of the theory of depolarization and for useful relations involving the use of polarized primary light.

TABLE V
DEPOLARIZATION FACTORS FOR UNPOLARIZED PRIMARY LIGHT

Pure liquids	Benzene	Toluene
Doty and Kaufman	0.410	0.490
Peyrot ^a	0.420	0.480

Cellulose Acetate in Acetone			
Fraction	Molecular weight	1% solution	0.5% solution
27B	65,000	0.045	0.045
16B	90,000	0.029	0.028
8B	158,000	0.017	0.019
Unfractionated	90,000	0.029	

^a P. Peyrot, *Compt. rend.*, **203**, 1512 (1936).

Besides determining molecular weights of large molecules by absolute measurements of light scattering as just described, it is also possible to deduce the distribution of molecular weights in high polymers by calibrative methods of light scattering. Morey and Tamblyn⁷⁴ determine the molecu-

⁷³ R. W. Wood, *loc. cit.*, p. 341.

lar weight distribution in a heterogeneous polymer by relating the mass of polymer precipitated on the addition of a precipitating agent to the solution to the amount of precipitant added. The precipitated polymer is estimated by the light it scatters. Since much preliminary calibration is required, the method is best adapted to repeated studies of a given type of polymer. The calibration for the type having once been established, new distributions of molecular weight caused, for example, by changes in the methods of synthesis, blending, etc., can be deduced with relative rapidity. The original article should be consulted for details.

E. PREPARATION OF SOLUTIONS FOR LIGHT-SCATTERING EXPERIMENTS

Since the intensity of scattering varies as a high power of the diameter of the particles, it is clear that care must be taken to exclude even small concentrations of particles in the solution of larger size than those under study. The effect of large particles on the dissymmetry is particularly marked. The following suggestions on preparation of the solvents and solutions are given by Mark (Part I, Chap. VIII), and in the articles of Doty, Mark, Zimm, and coworkers. It is pointed out that no complete prescription can be made at present and that standardization of procedure awaits the collection of further data.

Two types of impurities require attention.

(a) *Low Molecular Weight Impurities in Solvent and Polymer.*—Solvents should be carefully distilled until the boiling point, density, and refractive index do not change on further purification. Solvents like dioxane or formic acid, which readily pick up water, must be kept carefully dry, since small amounts of water change the refractive index appreciably. The polymer is advantageously dissolved at a concentration of 2-3% in the solvent in which the measurements are to be made, and purified by precipitation with a volatile precipitant. The precipitate is filtered and washed with the precipitant, and slowly dried at low temperature *in vacuo*, quick drying leading sometimes to the production of a horny material which dissolves with difficulty. The precipitation should be repeated as required; some polymers, such as polystyrene, are relatively easily freed from traces of monomer, catalysts, etc.; others, such as nylon and rubbery copolymers, retain impurities tenaciously.

(b) *Coarse Impurities of Solvent and Polymer.*—Coarse impurities in solvent and solution can be detected by Tyndall scattering and observable Brownian motion on examination of the illuminated liquid by means of a lens at a small angle to the direction of the primary beam. Large particles stand out as shining specks against a darker background. Dust in solvents

²⁴ D. R. Morey and J. W. Tamblin, *J. Applied Phys.*, **16**, 419 (1945).

can be removed by filtering under pressure through Corning fine sintered glass filters (about $15\ \mu$ pore size). Dust in polymers is relatively easily removed by precipitation, washing, resolution, and filtration through fine sintered filters. Haze, of organic origin, is often caused by chemical and structural heterogeneity of the polymer, and is sometimes difficult to remove. A pressure-type bacteriological filter (*e. g.*, Selas No. 4, *Selas Company*, Philadelphia) may suffice. A comparatively successful way of reducing haze seems to consist in precipitating the polymer three or four times, using different solvents in each resolution and also changing the precipitant.

An important factor in the choice of solvent for scattering experiments is the difference in refractive index between solvent and solute. The difference in refractive index between pure solvent and a 1% solution of the polymer should exceed 0.0005. The lowest molecular weight measurable is determined by this quantity.

4. Higher Order Tyndall Spectra

Only brief reference can be made to some of the methods recently used by LaMer and coworkers and by others for the determination of particle sizes greater than about $200\ m\mu$. These methods have proved useful in studying the growth of colloidal particles. An article by LaMer⁵⁹ contains a résumé of the theory and practice of these methods. If a dispersion of transparent dielectric particles of uniform size greater than about $200\ m\mu$ is illuminated by white light, the laterally scattered light shows a pattern of maxima and minima of intensity, whose number and angular position depend on the particle size, the ratio of the refractive index of the particle to that of the medium, and the wave length of the light. The angular sequence of colored maxima constitutes a *Tyndall spectrum*, and the different maxima of a particular color form, respectively, the first and higher order Tyndall spectra. The position of these orders can be calculated from Mie's theory of scattering by dielectric spheres of uniform size⁶⁴ and the calculated positions of the maxima compared with experiment to evaluate the particle size; or an empirical calibration can be made with particles of known size as found, for example, by sedimentation. A table of positions of higher order red Tyndall spectra for spherical particles of radii from 200 to $550\ m\mu$ and refractive ratio 1.44 (sulfur sols) is given by Johnson and LaMer.⁶⁴

The observation of Tyndall spectra requires a high degree of uniformity in particle size, and can be used as a criterion of monodispersity. LaMer⁵⁹ mentions an example in which equal quantities of two sols, whose particles differed by 2% in radius, each separately showing well-defined Tyndall

spectra, on mixing yielded sols which did not show the criterion of well-defined colored bands.

Figure 34 illustrates a simple apparatus for determining the positions of the orders.⁶⁴ An intense parallel beam of white light from the source, *B*, a General Electric projection bulb of 108 watts, is produced by lens *L*, and a narrow rectangular pencil, defined by slit *S*, $\frac{1}{32}$ by $\frac{1}{2}$ inch, is passed into the suspension, contained in a crystallizing dish, *D*, 8 cm. in diameter.

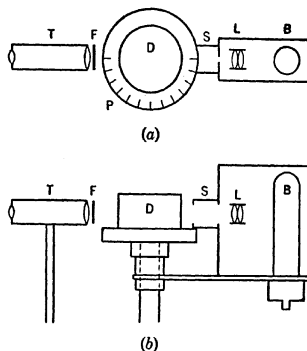


Fig. 34.—Apparatus for measuring higher order Tyndall spectra: (a) plan; (b) elevation.⁶⁴

The scattered light is observed through a small telescope, *T*, with a cross-hair in the focal plane of the eyepiece. The Tyndall orders are most distinct when the scattered light vibrates with the electric vector perpendicular to the plane containing source, particle, and observer, and a piece of Polaroid *F* is mounted in front of the object glass of the telescope so as to transmit such light. The light source can be rotated around the axis of the dish, and the angle between the incident beam and the telescope is read from the divided scale, *P*. With this apparatus the position of an order can be determined to within 1° .

Quantitative data of the ratio of red to green light can be made by replacing the telescope with a photometer. Johnson and LaMer used a Luckiesh brightness meter in conjunction with red (Wratten No. 29) and green filters (Wratten No. 58).

5. Application of Turbidimetric Methods to Analysis and Counting of Larger Particles

Turbidimetric methods have been used considerably, perhaps especially by biochemists and in certain technologies, in analytical procedures involv-

ing the production of fine precipitates, and in counting small particles such as microorganisms and fine inorganic suspensions. These methods, like colorimetric methods, are calibrative, and limited to low concentrations, the measurement involving either the direct comparison of the sample with a standard, or the translation of a reading for the sample into concentration from a previously made "working curve" showing the relation between readings and concentrations. The sensitivity of turbidimetric methods of analysis is of the same order as that of colorimetric methods: dilutions of 1×10^5 or 1×10^6 are commonly measured, and some exceptionally sensitive reactions are known, such as the precipitation reaction between silver mercuric cyanide and acetone, which permits estimation of one part of acetone in 10^8 of diluent. Methods can often be adapted to the analysis of very small volumes of solution.

A. NEPHELOMETER

In the nephelometer, the suspension is placed in a test tube illuminated from the side, and the light scattered axially along the tube is observed. The most accurate visual instruments employ the principle of the Duboseq colorimeter (Fig. 19) with the modifications of substituting a test tube with transparent walls and blackened flat bottom for the black walls and transparent bottom of the colorimeter. The standard and sample tube are placed side by side in a uniform beam, secured by rendering parallel the light from a concentrated filament lamp. The standard is usually prepared by adding the precipitating reagent under the same conditions as for the sample, to a system as nearly as possible identical with the unknown, containing a known concentration of the analyte and not very different from that expected in the sample. This is placed in one cup and the sample in the other; the length of the illuminated standard column is set at a suitable value, and that of the other adjusted until the two halves of the field seen in the ocular seem equally bright. The reading is made by adjusting the sample alternately from above and below until a faint shadow of the same intensity appears first on one side then on the other of the field; the operation is repeated several times and the mean of all the readings is taken. With some instruments and with some systems, the concentrations of suspended matter in the two tubes are inversely proportional to the lengths at photometric match, but this relation cannot be taken for granted and must be verified for any specific analysis. If the relation is not found, an empirical calibration curve can be constructed and, for accurate determinations, the calibration should be made for every concentration used in the standard.

Before analytical measurements are made, the apparatus should be checked for symmetry of illumination and matching of the two test tubes. The same stable suspension is placed in the two tubes, adjusted to the same length, and the position of the light source adjusted with respect to the instrument for equality of the fields. The same side of the tubes should always be presented to the light, and a mark made outside the illuminated portion of the tube to insure this position. Balance having been achieved, the tubes are interchanged; if the fields are still matched the illumination is symmetrical, if not the source is readjusted and the operation repeated until no change is seen on interchanging the tubes. It may be that no such position of the source can be found, in which case, if the two suspensions are truly the same, either the tubes do not match, or there is a displacement of some element in the optics of the instrument. Nonmatching tubes can be recognized by the fact that the inequality in the fields will reverse its sign when the tubes are interchanged, and in that case a correction can be made. If the inequality remains the same irrespective of interchange of the tubes, there is a maladjustment in the optics of the instrument, which should be returned to the maker for readjustment.

Visual nephelometry can be made a precise method of analysis. The subject is treated in detail by Yoe,⁷⁵ to which reference should be made for instrumental minutiae and for chemical details for specific determinations in inorganic, organic, and biochemical analysis. Kleinmann has designed an attachment which permits precise nephelometric determinations with 3 to 5 cc. of sample. This is substituted for the normal tube, holding about 17 cc., in a macro instrument.⁷⁶

Success in turbidimetric analysis, whether by subjective or objective methods, depends at least as much on the reproducibility and other characteristics of the precipitate as on the degree of instrumental perfection. The turbidity of the solution must obviously remain constant over the period of observation and, if secular changes occur, the interval of time between the addition of the reagents and the measurement must be standardized. Flaky precipitates are unsuitable. The addition of a protective colloid, starch, gelatin, or the like, may facilitate in the production of uniform suspensions, particularly of inorganic compounds like silver chloride and barium sulfate.

B. PHOTOELECTRIC NEPHELOMETERS

As in colorimetry, the trend in turbidimetry is toward substituting objective for subjective methods. Most commercial fluorimeters can be easily adapted to the measurement of scattered light. The simplest ar-

⁷⁵ J. H. Yoe, *Photometric Chemical Analysis*. Vol. II, Wiley, New York, 1929.

⁷⁶ H. Kleinmann, *J. Lab. Clin. Med.*, 12, 629 (1927).

rangement, a direct-deflection, photoelectric nephelometer is illustrated in figure 35.⁷⁷

The source, *S*, a 32 candle power, 6-8 volt automobile lamp, with terminals soldered to the leads from the battery, lens *L* and the "Insol" glass beaker, *B*, in which the precipitate is produced are enclosed in a blackened housing, which also contains thermostated pipettes, not shown in the diagram, for the addition of reagents. The scattered light is received by the Photronic cell, *Ph*, connected directly to a sensitive galvanometer with a resistance of 500 ohms and a current sensitivity of about 10^{-9} amp. per mm.

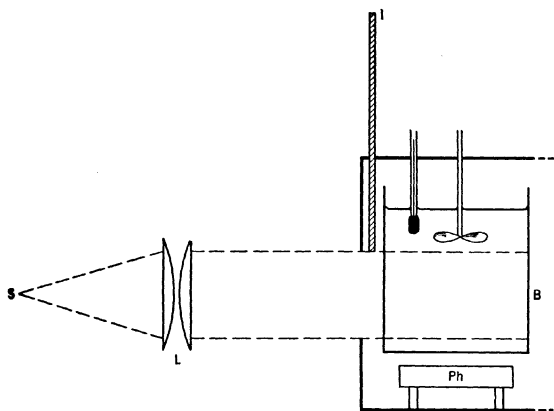


Fig. 35.—Simple photoelectric nephelometer.⁷⁷

Other circuits, such as those described in Chapter XXI, Section III, could be used, preferably a "zero-potential" arrangement such as that of Brice. At the relatively feeble intensities of scattered light, however, substantial linearity of response to intensity is to be expected in the simple direct circuit with an external resistance of about 500, although this should be verified over the range of intensities encountered. Constancy of the light source was secured by Greene by operating it from a large battery at a reduced voltage of about 5.1, accurately checked at the start of each measurement by a potentiometer, and by preconditioning a new lamp for 10-20 hours at 6 volts across the filament. An automobile lamp supplied from a step-down transformer, fed from the mains through a constant-voltage transformer, would probably be adequately constant for many measurements of this type.

⁷⁷ C. H. Greene, *J. Am. Chem. Soc.*, **56**, 1269 (1934).

The readings of any direct-deflection photometer, whether used for colorimetry, fluorimetry, or turbidimetry, should be compared with those furnished by an invariable standard, to eliminate the effect of the inevitable day-to-day variations in sensitivity. In the instrument described in figure 35, the standard is a piece of ground glass firmly cemented at an angle in a beaker similar to *B* so as to reflect diffuse light into the photocell. In use, the ground glass is immersed in water. The turbidity of a precipitate is then expressed as the ratio of the corresponding deflection to that for the

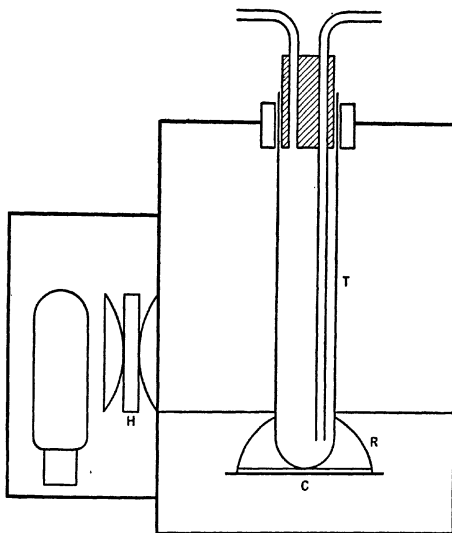


Fig. 36.—Photoelectric nephelometer.⁷⁸

standard. As an index of the sensitivity of this apparatus, Greene⁷⁷ was able to study with satisfactory reproducibility the growth of suspensions of silver chloride having a concentration of about 2.5 times that of a saturated solution of silver chloride in pure water.

A photoelectric nephelometer on the same principle,⁷⁸ in which the vessel containing the suspension is an ordinary test tube, is shown in figure 36. The source is a 100-watt concentrated filament lamp, supplied from the a.-c. mains through a Sola constant-voltage transformer of 120 watts, whose output of 115 volts is adjusted to any desired voltage by a 1-amp.

⁷⁸ N. Underwood and A. H. Doermann, *Rev. Sci. Instruments*, **18**, 665 (1947).

Variac or a 3-amp. Powerstat (*Superior Electric Company*). The condensing system is of approximately $f/1.5$ aperture ratio, with a plate of heat-absorbing glass, H (for example, Corning Aklo glass), between the lenses. About 9.5 cc. of the suspension, in tube T , mounted in a blackened box, is illuminated. The scattered light is reflected by R on to a selenium barrier layer cell, C (*Selenium Corporation of America*), at the bottom of the sample tube. The photocurrent is measured as the direct deflection of a galvanometer with an enclosed illuminated scale, with a critical damping resistance of about 40 ohms and a sensitivity of 1.9×10^{-7} amp. per division (*G. M. Laboratories, Chicago*). The light scattered by a suspension of *Escherichia coli* containing 5×10^7 per cc. produced a photocurrent of 1.2×10^{-8} amp. and reliable readings can be made from 10^7 to 10^9 organisms per cubic centimeter.

If a large range of concentrations is to be studied in a direct-deflection turbidimeter, the light scattered by the higher concentrations from a primary beam of sufficient intensity to allow measurement of the lower concentrations would send the galvanometer deflection off scale unless the sensitivity were decreased. Suitable reductions in intensity with overlapping calibrations in the various ranges can be made, but a more convenient procedure is to keep the intensity constant at a value adequate for the low concentrations and reduce the sensitivity of the measuring apparatus. This can be done by means of a decibel step-type attenuator (*Clarostat*) which presents a constant impedance to the photocell and to the galvanometer as the sensitivity is changed.

Photoemissive tubes and electronic amplification can also be used in nephelometers.⁷⁹ In the instrument of Krebs *et al.* stability is achieved with the aid of an electronic power supply which stabilizes both the lamp and the amplifier. The readings are obtained as direct deflections on a microammeter with a range of 0 to 200 μ a. Yeast cultures containing $1-10 \times 10^6$ organisms per cc. are within the range of the instrument, and pantothenic acid from 0.01 to 0.06 μ g. per cc. was determined by using the growth of *Lactobacillus casei* as the measure of the vitamin.

C. TRANSMISSION TURBIDIMETERS

Solutions sufficiently turbid to decrease the transmission by about 5% or more can be measured in a spectrophotometer or photometric colorimeter. Illustrative of a good type of design for a photoelectric transmission turbidimeter and colorimeter is the null instrument of Baier.⁸⁰ This instrument

⁷⁹ R. P. Krebs, P. Perkins, A. E. Tytell, and H. Kersten, *Rev. Sci. Instruments*, **13**, 229 (1942).

⁸⁰ J. G. Baier, *Ind. Eng. Chem., Anal. Ed.*, **15**, 144 (1943).

employs a phototube and amplification, but the indications of the current meter are used only to show a deviation from photometric balance, the actual reading being made in terms of the motion of a calibrated wedge which is adjusted to restore balance. Strict linearity of the photocurrents with the intensity of the light and of the output with the input signal in the amplifier are therefore not of vital importance. Several arrangements in which the readings are made in terms of the indications of a rebalancing potentiometer are noted in this and the preceding chapter.

The instrument is shown in figures 37, 38, and 39. In figure 37, two photocells, C_1 and C_2 , are illuminated by the same source, S ; fluctuations in which affect both cells equally and have no effect on the null reading. If an absorbing sample is introduced in front of one of the cells, the change in intensity falling on one compared with that on the other causes a change in the grid potential of the amplifier and a change in the reading in the microammeter in the plate circuit from the null position. This change is compensated for by diminishing the amount of light reaching the other cell, by partially covering an entrance aperture by the calibrated wedge, W_1 , until the microammeter points again to the null position (Fig. 39). Diffusion disks of flashed opal glass are used to produce even illumination of the wedges and to cause the beams entering the photocells to cover the whole cathode surface.

The null point is found by introducing pure solvent in test tubes into holders T_1 and T_2 , setting calibrated wedge W_1 at the position corresponding to 100% transmission, and adjusting the similar wedge W_2 so as to cause the meter to register the arbitrary null reading, say 100 μ a. The unknown sample is then substituted for the solvent in T_2 and W_1 is moved to restore the null reading. 1 cc. of sample is sufficient, in 10×75 mm. serological tubes or $6 \times 6 \times 75$ mm. square tubes. F_1 and F_2 are holders for filters. The sensitivity of this apparatus can be increased by concentrating the beams by means of lenses.

The circuit is shown in figure 38. The amplifier and phototube voltages are regulated by a VR 105-30 regulator tube, so that changes in line voltage have a negligible effect on the circuit, and the vacuum tubes are run at lower than their rated potentials. The elements of the circuit are: V_1 = RCA 919; V_2 = RCA 917; V_3 = 38; V_4 = VR 105-30; V_5 = 80; R_1 = 7000 ohms, 25 watts; R_2 = 7500 ohms, 75 watts; R_3 = 2000 ohms, 25 watts; R_4 = 150,000 ohms protective resistance; M = 0-200 microammeter, Weston model 301; C_1 = 16 μ f. electrolytic condenser (negative terminal insulated from ground); T_1 = power transformer (Thordarson T13R11), filament winding to 38 amplifier reduced from 6.3 to 4.0 v. by series resistor; T_2 = 115-5 step-down transformer (U.T.C. S-54) for lamp illuminating photocells; S = switches in primaries of transformers T_1 and T_2 . Contact pins 3 and 7 of VR 105-30 are inserted in a. c. power input lead as a safety measure; CH = choke (Thordarson T68C08).

The voltage across the resistors R_2 and R_4 is maintained at 105 v. by the regulator

tube VR 105-30. The end of R_3 is 22 v. below ground; this is the voltage of the cathode of the 917 phototube, while the anode of the other tube is adjusted to almost the same potential above. At no illumination, or at equality of illumination, the common lead from the photocells to the control grid of the amplifier tube is substantially at ground potential. The plate of the amplifier is run below the rated value, at 25 v., to maintain stability. The screen grid is set at a sufficient positive potential for the desired sensitivity, and is variable in steps of 5, 10, and 15 v.

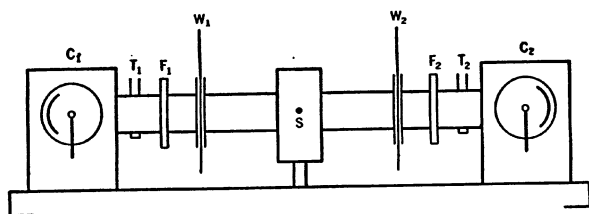


Fig. 37.—Null-reading photoelectric photometer.⁸⁰

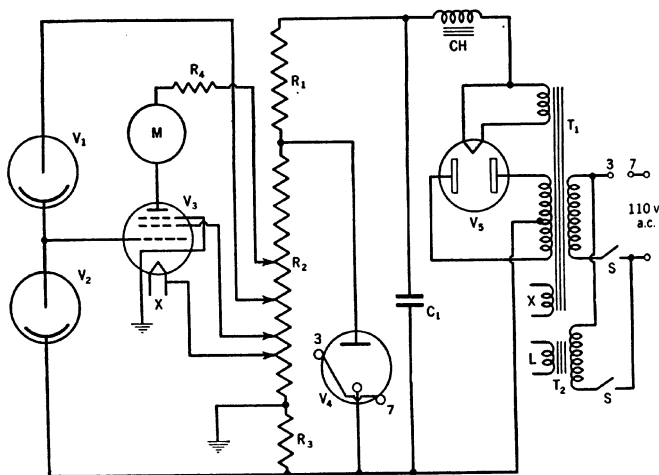
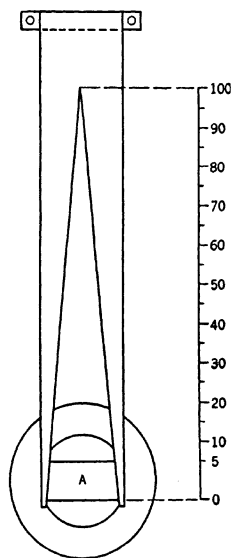


Fig. 38.—Circuit for null-reading photometer.⁸⁰

The construction of the wedge is shown in figure 39. A is a trapezoidal window area admitting light to the filter, sample, and photocell. The area of the trapezoid is $\frac{1}{2}(a + b)h$, where the base, a , top, b , and altitude, h , are in the ratio of 21:19:10, and h is $\frac{3}{8}$ inch. The scale is drawn by computing the fraction of the window area left after moving the wedge from position 100 to other positions, and is linear except near the lower end. Window, wedge, and scale must be made with precision.

An interesting principle in the turbidimetry of very slightly turbid media is described by Silverman.⁸¹ In figure 40 G_1 and G_2 are two grids, consisting of alternate bars and openings of equal width. The bars are 2 inches long and $\frac{3}{16}$ inch wide, and, to secure identity as nearly as possible, the two grids are milled simultaneously, from $\frac{3}{32}$ -inch brass. Each grid contains 4 bars, each ground to a fine feather edge, and the whole is blackened to minimize diffraction and reflection. G_1 is rigidly mounted on the base plate, while G_2 is placed in a holder capable of lateral and forward and backward motions, and also a few degrees of rotation on each side of the vertical.

Fig. 39.—Construction of wedge and calibrated scale for regulating light and measuring light incident on photocell.⁸⁰



Both grids are mounted with the feather edges away from the light source. Barrier layer photocells (Weston Photronic or Electrocell cells) are connected in the circuit of Brice (this chapter, page 1418), the rheostat being 200 ohms, and the meter, M , a 0–50 microammeter, Weston No. 430. L is composed of two 4-inch projection objectives (Spencer Delineascope) sliding in a tube for focus adjustment; and the two planoconvex lenses, 6 cm. diam., near the source, S , form a condenser. Source is a 32-candle power automobile lamp, operated from a voltage-regulating transformer

⁸¹ S. Silverman, *Rev. Sci. Instruments*, 12, 77 (1941).

(*Raytheon*) and step-down transformer. The whole instrument is enclosed in a box with a convenient opening to admit the sample.

The grid, G_1 , is evenly illuminated by the beam from the condenser, and an image of unit magnification is produced at G_2 by adjusting L . G_2 is now adjusted so that the bright parts of the image of G_1 fall on the bars of G_2 and no light reaches photocell P_1 . Usually insufficient correction of the lenses causes some stray light to reach P_1 ; this is balanced out by the current from P_2 , by adjusting the rheostat.

When a turbid sample, in an absorption cell, is introduced at X , the image of G_1 is no longer sharp, and light scattered into the shadows of the

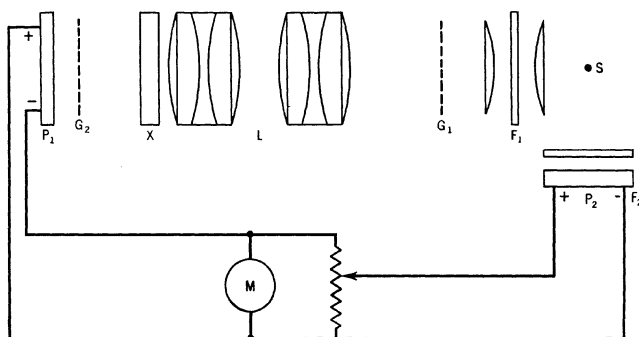


Fig. 40.—Turbidimeter according to Silverman.⁸¹

bars of G_1 passes the openings of G_2 to the photocell, P_1 . For small turbidities, the intensity of the light reaching P_1 is proportional to the concentration of the scattering particles. A difference of several microamperes is indicated by a difference between distilled and tap water, accurate to about $0.5 \mu\text{a.}$ and the higher end of the range over which the instrument has been used is 200–300 times the turbidity of tap water.

By substituting a high-sensitivity galvanometer for the microammeter (Rubicon 3303 HH, 1100 ohms, 5×10^{-9} amp. per mm.), counts on small micellar organisms such as *Colpidium campylum* at a concentration of 1000 to 17,000 per cc. have been made. Other possible applications are for white blood or bacterial counts, or for the measurement of feeble fluorescent light.

Transmission turbidimetry is used in the quick routine analysis of water supplies for insoluble matter and in the determination of sulfate in boiler

waters. The simplest of these devices is the *Jackson Turbidimeter*. The turbid medium is poured into a graduated cylinder until the flame of a candle looked at axially through the tube can no longer be perceived. Comparison is made with standards of powdered silica in water.

A more accurate instrument of this type is the *Sulfur Turbidimeter*⁸² of Parr and Stayley used in determining the sulfur content of coal, oils, boiler waters, etc. The suspension of barium sulfate is placed in fixed tube *A* (Fig. 41), provided with an optical window at the lower end, and plunger *B* is moved by means of the rack until the eye at *E* no longer sees the filament of

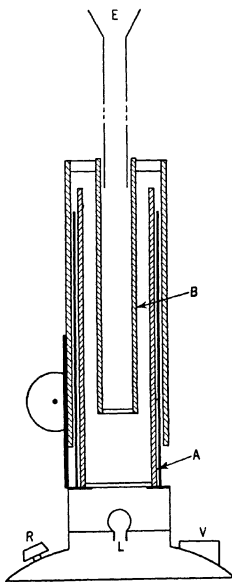


Fig. 41.—Parr turbidimeter (diagrammatic).

the 2 candle power lamp, *L*, supplied by two or three dry cells. The distance between the lamp and eyepiece is constant; the distance between the bottoms of *A* and *B* is recorded on a scale. The lamp is run at a constant voltage, indicated on the voltmeter, *V*, and regulated by rheostat *R*. Estimations are made from working curves constructed from known samples.

⁸² S. W. Parr and W. D. Stayley, *Ind. Eng. Chem., Anal. Ed.*, **3**, 66 (1931).

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POLARIMETRY

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Symbols Used in Text and Figures

<i>A</i> , analyzer	<i>T</i> , transmission (<i>OT</i> , direction of maxi- mum transmission through analyzer)
<i>B</i> , brightness (<i>B_s</i> , of light source or optical field; <i>B_{ea}</i> , equality in two fields)	<i>V</i> , vibration; direction of vibration (in abbreviation of <i>OV</i>)
<i>D</i> , diaphragm	<i>V_o</i> , direction of vibration of beam emerg- ing from polarizer
<i>E</i> , extinction (<i>OE</i> , direction of extinction in analyzer)	<i>V₁</i> , after traversing optically active substance
<i>G</i> , glass	<i>V'₁</i> , after traversing optically active substance which absorbs or scatters light
<i>I</i> , intensity	<i>V_t</i> , vibration of linearly polarized beam incident upon analyzer, without spec- ification of changes in <i>V_o</i>
<i>I_i</i> , of beam incident upon analyzer	<i>a</i> , amplitude (meanings of subscripts to <i>a</i> are analogous to those of subscripts to <i>V</i>)
<i>I'_t</i> , after passing through light-absorbing substance	<i>b</i> , layer thickness
<i>I_o</i> , emerging from analyzer in absence of optically active substance	<i>i</i> , angle of incidence
<i>I₁</i> , emerging in presence of optically ac- tive substance	<i>n</i> , refractive index
<i>I'_o</i> , <i>I'₁</i> , same if optically active substance absorbs or scatters light	<i>r</i> , angle of reflection (as subscript to <i>R</i> , re- sultant ray)
<i>L</i> , lens	<i>t</i> , time
<i>M</i> , mirror	<i>v</i> , velocity of light (<i>c</i> , in <i>vacuo</i>)
<i>N</i> , nicol, general meaning (see page 1517)	<i>x</i> , optic axis of uniaxial substance
<i>P</i> , polarizer	<i>α</i> , angle of rotation due to natural rotatory power
<i>Pr</i> , prism	
<i>Pt</i> , polarimeter tube	
<i>Q</i> , quartz	
<i>R</i> , light ray	
<i>S</i> , light source	

- β , angle at apex of quartz wedge, cut perpendicular to optic axis
 γ , angle between optic axis of uniaxial crystal and direction of vibration of incident linearly polarized light beam
 δ , angle at apex of birefringent crystal wedge
 ϵ , one-half of the half-shade angle
 ζ , angle by which the position of half-shade equality deviates from position of crossed nicols (ζ' , increment of ζ , due to loss in intensity by reflection)
 η , angle between direction of transmission of analyzer and direction of vibration of incident beam
 θ , angle of rotation due to linear dichroism
 λ , wave length (λ_E , extinct by analyzer; λ_{E_1} , equally bright in two spectra compared after passage of light through analyzer)
 ν , frequency
 π , 180°
 ρ , angle of mechanical rotation (ρ_P , of polarizer; ρ_A , of analyzer; ρ_{SP} , of spectrograph)
 σ , angle between direction of extinction of analyzer and direction of vibration of incident beam
 τ , range of angles of rotation of optically active substance between 6500 and 4500 Å.
 φ , phase difference
 χ , angle of rotation due to magnetic rotatory power (Faraday effect)
 ω , numerical reading on circular scale of polarizer or analyzer
 \longleftrightarrow , vibration in the paper plane or in perspective
 \times , vibration perpendicular to paper plane
 $\overset{x}{\longleftrightarrow}$, optic axis in the paper plane or in perspective
 $x \bullet$, optic axis perpendicular to paper plane
 $\bigcirc \rightarrow \leftarrow \bigcirc$, direction of propagation in paper plane or in perspective
 \oplus , direction of propagation perpendicular to paper plane and toward reader
 \bigcirc , direction of propagation perpendicular to paper plane and away from reader

I. DEFINITIONS

Polarimetry deals with the quantitative investigation of all optical phenomena involving polarized light. It has become customary, however, to restrict the term "polarimetry" to quantitative investigations of the two following changes that may occur in polarized light when it interacts with optically anisotropic matter: (a) a change in the direction of vibration, *i. e.*, a rotation of a linear vibration perpendicular to the direction of propagation; and (b) a change in the state of vibration, *i. e.*, a transition of a linear vibration into an elliptic or circular vibration or vice versa. Substances that produce neither change are called optically *isotropic*, which implicitly defines the term *anisotropic*. A large number of phenomena are thus excluded in the customary definition of polarimetry, although their study calls for polarimetric methods, *e. g.*, the polarized fluorescence of dye solutions irradiated with unpolarized light. The organic chemist, however, is accustomed to an even narrower definition of polarimetry which excludes everything except quantitative investigations of the change in the direction of vibration of linearly polarized light during its passage through optically anisotropic liquids or solutions. This definition of polarimetry arises from the fact that, when polarimetry was developed a

little over a hundred years ago, the optical phenomenon just mentioned was the only one involving polarized light known in organic liquids or solutions. The present chapter is limited to a discussion of the fraction of polarimetry defined by this tradition.

The change in the direction of vibration of linearly polarized light during passage through anisotropic matter is called "rotatory polarization," or "optical rotation" if the effect is due to anisotropic refraction. If it is caused by anisotropic absorption or anisotropic scattering, it should not be called "optical rotation," but "dichroism" (see page 1505). Substances

that cause rotatory polarization are said to exhibit rotatory power. Substances that possess a "natural" rotatory power are called "optically active." A rotatory power may also be induced in a naturally isotropic substance by a magnetic field (Faraday effect). We shall deal primarily with the measurement of natural rotatory power.

The effect of rotatory power is shown in figure 1. A beam of linearly polarized light traveling perpendicular to the plane of the paper toward the reader intersects the plane at O . The beam vibrates along $-V_0OV_0$, but will vibrate along $-V_1OV_1$ or $-V_1'OV_1'$ after traversing an optically active substance. Rotation α is positive if it is clockwise ($-V_0OV_0 \rightarrow -V_1OV_1$), negative if the direction of the vibration is changed to $-V_1'OV_1'$. Substances that cause a positive rota-

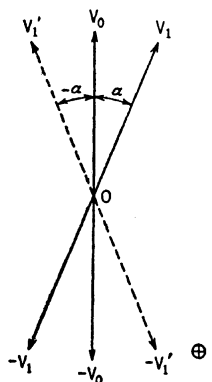


Fig. 1.—Optical rotation.

tion are called dextrorotatory or dextrogyrate, and substances causing a negative rotation, levorotatory or levogyrate.

In the following, the direction of vibration will be indicated by a single symbol only. V_0 , for example, will stand for $-V_0OV_0$. In order to keep the figures as simple as possible, only half of a vibration will be reproduced, for instance, OV_0 instead of $-V_0OV_0$. This half represents the amplitude, a , of a vibration. The value of a is equivalent to the square root of the light intensity, I .

Most authors use the terms "plane polarized," "elliptically polarized," and "circularly polarized." We use the term "linearly polarized" instead of "plane polarized" because the principle of projection involved is then identical for the three types of vibration. The terminology is essentially the same as that introduced by Brewster,¹ who distinguished between rectilinear, elliptical, and circular polarization.

¹ D. Brewster, *A Treatise on Optics*. 1st Am. ed., Carey, Lea, and Blanchard, Philadelphia, 1833, p. 191.

II. PHYSICAL OPTICS OF ROTATORY POLARIZATION

Rotatory polarization in the case of optical activity was analyzed by Fresnel.² Linearly polarized light may be considered as the resultant of two coherent circular components of opposite sense of rotation, the *d*- and the *l*-component. The term *coherent* implies that the two components are able to interfere with each other. Furthermore, it is assumed that the velocities of the two components in the optically active substance differ. Figure 2 illustrates the case of an isotropic liquid.

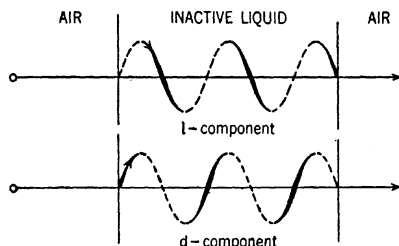


Fig. 2.—Schematic resolution of a beam of linearly polarized light into two circular components. Optically inactive liquid.

The actual phenomenon is visualized by a superposition of upper and lower drawings. Vibrations behind the plane of the paper are indicated by broken lines.

A linearly polarized beam of monochromatic light travels along the plane of the paper in the direction of the arrow. Rotating around the direction of propagation are the two circular components into which the beam is assumed to be resolved after it enters the liquid. The velocity of the two components in the liquid, $v_d = v_l = v_2$, differs from that in air, v_1 , according to:

$$v_1 : v_2 = n_2 : n_1 \quad (1)$$

where n_1 is the refractive index of air and n_2 that of the liquid. The refractive index is defined by:

$$n = c/v \quad \therefore n_1 = c/v_1 \quad \text{and} \quad n_2 = c/v_2 \quad (2)$$

where c is the velocity of light in a vacuum. Since the frequency, ν , the number of vibrations per second, is constant and since:

$$\nu = v/\lambda \quad (3)$$

it follows that:

$$\lambda_1 : \lambda_2 = v_1 : v_2 = n_2 : n_1 \quad (4)$$

where λ_1 and λ_2 are the wave lengths in air and in the liquid, respectively. Consequently, the light waves are contracted in the liquid.

² A. Fresnel, *Ann. chim. phys.*, 28, 147 (1825).

In figure 3, the isotropic liquid is replaced by an optically active one. This time, $v_d \neq v_l$, i. e., the optically active liquid has two refractive indices, n_d and n_l . This implies that λ_d differs from λ_l and that the planar projections of the two components will get out of phase, as shown in figure 3. Before discussing the significance of this getting out of phase, we consider the recombination of the two components leaving the isotropic liquid. In figure 4, the direction of propagation is perpendicular to the paper plane. We study the recombination, in a given plane perpendicular to the direction of propagation, during one half period, i. e., during the time required by the light to advance by one half wave length, and we divide this period into four intervals, defined by the times t_0 to t_4 . Evidently the resultants of the circular components fall on a straight line and the recombination results in a linear vibration, V_1 , in the same direction as V_0 . In figure 5, the case of an optically active liquid, the planar projections of the two circular components have reached a

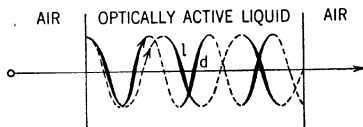


Fig. 3.—Schematic resolution of a beam of linearly polarized light into two circular components. Optically active liquid.

phase difference, φ , of $\pi/4$, i. e., 45° upon arriving in the plane considered, the d -component being ahead of the l -component. The recombination leads again to a linear vibration, V_1 , but the latter is rotated by 22.5° with respect to the initial vibration, V_0 . The rotation, α , amounts therefore to one-half of the phase difference, φ , of the planar projection of the two circular components. It is evident from the preceding equations and figures that:

$$\varphi = [(n_d - n_l)2\pi b]/\lambda_0 \quad (5)$$

where b is the layer thickness and λ_0 the wave length in a vacuum. Consequently, the rotation can be expressed in terms of the difference of n_d and n_l of the optically active medium:

$$(n_d - n_l) = \alpha\lambda_0/\pi b \quad (6)$$

This expression is seldom used, although the difference $(n_d - n_l)$, as the cause of the rotation, may be more significant than α .

The rotation is positive (Fig. 5) if $n_d < n_l$, i. e., if $(n_d - n_l)$ is negative and vice versa.

Since φ is proportional to the layer thickness, b , the degree of rotation, α , is also proportional to b , a regularity incorporated in equations (5) and (6), and proved, experimentally, by Biot.³

³ J. B. Biot, *Mém. Prem. classe Inst. France*, 13, 218 (1812).

According to the writer, another formal analysis of rotatory polarization is possible. It is, for some purposes, more advantageous. The arrows shown in figure 6 give the direction and the relative magnitude of the electric vector of linearly polarized light, which travels in the direction of the horizontal arrow and vibrates in the plane of the paper. The electric vector maintains its azimuth, but varies harmonically between a maximum positive and negative value. An attempt is made in figure 7 to express a circular vibration by a vector quality. To that effect, the circular vibration is considered as equivalent to the resultant of two orthogonal coherent linear vibrations which have a phase difference of 90° (see pp. 1512-1513). It follows from the figure—which shows only the formation of the

¹See G. Meslin, *Compt. rend.*, 152, 1666 (1911).

d-component—that rotatory polarization can be characterized as the result of a difference in the rate of rotation of two electric vector resultants of opposite sense of rotation. This concept leads at once to an easy understanding of the fact (page 1513) that a right circular or left circular vibration, important for the investigation of the Cotton effect (page 1501), can be obtained from linearly polarized light by sending the light beam through a linearly anisotropic substance (pp. 1508 *et seq.*) which produces a phase difference of $n \times 360^\circ - 90^\circ$ and $n \times 360^\circ + 90^\circ$, respectively, between two coherent orthogonal linear vibrations, n being any integral number or zero.

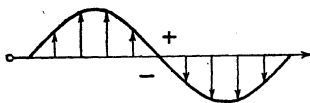


Fig. 6.—Linear vibration as the result of harmonic variation in the magnitude of a vector of constant azimuth.

which produces a phase difference of $n \times 360^\circ - 90^\circ$ and $n \times 360^\circ + 90^\circ$, respectively, between two coherent orthogonal linear vibrations, n being any integral number or zero.

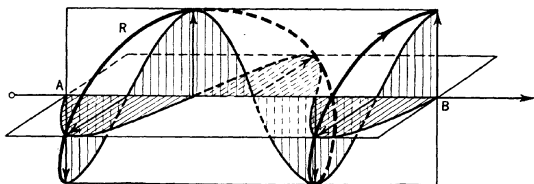


Fig. 7.—Circular vibration by rotation of a resultant vector of constant magnitude.

The arrows perpendicular to the horizontal direction of propagation show the electric vector of either of the two coherent orthogonal linear vibrations at the instant of maximum value. The helix, *i. e.*, circular vibration (heavy print), results from interference of the two linear vibrations due to their phase difference of 90° .

III. ROTATORY POWER OF ORGANIC MOLECULES

1. Pasteur's Principle

Pasteur⁶ discovered the principle which makes it possible to predict what molecules can be optically active. Each of the models of a right circular and of a left circular vibration in figure 2 is the mirror image of the other; they cannot be brought into coincidence around whatever axis one may turn them. Similarly, according to Pasteur, a compound will be optically active if its structure cannot be brought into coincidence with its mirror image. Such a "dissymmetric" structure does not have a plane of symmetry, though it may have an axis of symmetry. Pasteur arrived at his principle after discovering: (1) that optically active compounds can exist in two enantiomorphic modifications—"optical antipodes or isomers"—with a rotatory power of identical value but opposite sign; and (2) that crystals of optically active compounds may have a shape exactly like the mirror image of crystals of the respective antipode.

⁶ L. Pasteur, *Ann. chim. phys.*, **24**, 442 (1848).

Structural formulas which satisfy Pasteur's principle were given by van't Hoff⁷ and Le Bel,⁸ who conceived of organic molecules as three-dimensional structures, the four valences of carbon extending from the center to the corners of a tetrahedron.

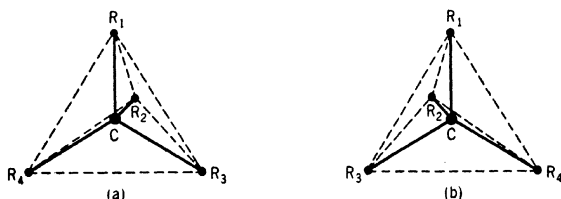


Fig. 8.—Van't Hoff's tetrahedral molecule and its theoretically simplest two optical antipodes. R_1 , R_2 , R_3 , and R_4 represent unequal substituents.

The simplest molecule which, according to van't Hoff, would be optically active, is symbolized in figure 8. The four substituents, R_1 , R_2 , R_3 , and R_4 , at the carbon atom, C, must all be different from each other in order that structures *a* and *b* cannot be brought into coincidence. A carbon atom thus substituted is called *asymmetric*.

Its presence is, however, not indispensable for optical activity. According

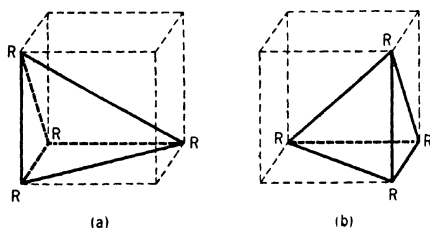


Fig. 9.—Tetrahedral model containing four identical substituents, R . Optical activity is due to dissymmetric inequality of edges.

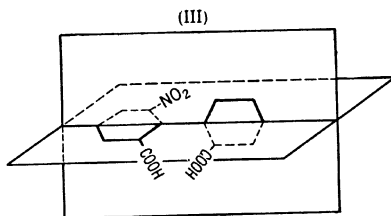
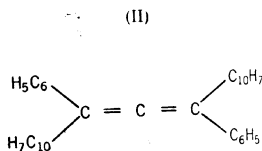
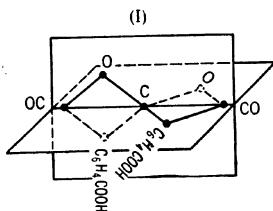
to Pasteur's principle, it would not be necessary to have four unequal substituents around the central carbon atom if the tetrahedron were deformed as in figure 9. A model of this type was actually found to be active toward Hertzian waves.⁹ An example for actual molecules is not yet known, but the interatomic distances and

⁷ J. H. van't Hoff, *La chimie dans l'espace*, Rotterdam, 1874; or *The Arrangement of Atoms in Space*, translated and edited by A. Eiloart, Longmans, Green, London, 1898.

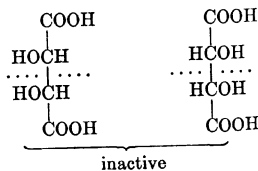
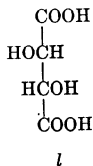
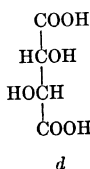
⁸ J. A. Le Bel, *Bull. soc. chim.*, 22, 337 (1874).

⁹ K. F. Lindman, *Ann. Physik*, 63, 621 (1920); 69, 270 (1922); 74, 541 (1924); 77, 337 (1925). See also B. Y. Oke, *Proc. Roy. Soc. London*, A153, 339 (1936).

changes in the diameter of the substituents are known to affect the optical rotation.¹⁰ According to Pasteur's principle, it is even unnecessary to have a tetrahedral structure. Absence of a coplanar structure may be the cause of dissymmetry, and of optical activity, of organic molecules. Some of the more recent representatives are shown below, *viz.*, a spiro compound (I),¹¹ an allylenic compound (II),¹² and a compound in which steric hindrance prevents rotation of critical parts of the molecule (III), *i. e.*, the formation of a coplanar structure.¹³⁻¹⁵



On the other hand, a molecule may contain several asymmetric carbon atoms without being dissymmetric. Tartaric and mesotartaric acid are classic examples.



¹⁰ See, for example, D. H. Brauns, *J. Am. Chem. Soc.*, **56**, 1421 (1934); **47**, 1285 (1925).

¹¹ For example, W. H. Mills and C. R. Nodder, *J. Chem. Soc.*, **117**, 1407 (1920); **119**, 2094 (1921). W. H. Mills, *Trans. Faraday Soc.*, **26**, 431 (1930).

¹² R. Maitland and W. H. Mills, *J. Chem. Soc.*, **1936**, 987.

¹³ W. H. Mills, *Chemistry & Industry*, **45**, 884 (1926). W. H. Mills and K. A. C. Elliott, *J. Chem. Soc.*, **1928**, 1291.

¹⁴ F. Bell and J. Kenyon, *Chemistry & Industry*, **45**, 864 (1926).

¹⁵ J. F. Hyde and R. Adams, *J. Am. Chem. Soc.*, **50**, 2499 (1928) and subsequent papers by R. Adams.

The structure of the former is dissymmetric. It exists in two optically active modifications. The structure of the latter is not dissymmetric; it is inactive. The structures are shown above in simplified planar projection,¹⁶ the broken line indicating the plane of symmetry.

2. Cotton Effect

Pasteur's principle defines the structure which a molecule must have in order to be optically active, but it cannot explain why an appropriate structure leads to optical activity. It is the aim of the modern theories to answer this question. The development of these theories was decisively stimulated by the discovery of the Cotton effect.

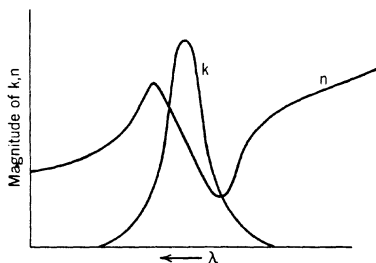


Fig. 10.—Variation of refractive index in the spectral region of an absorption band.

Refraction results from the superposition of a primary light wave entering the refracting substance and a secondary "scattered" wave created by the vibration of dipoles that are induced in the substance by the primary wave. The dipole vibration is a function of the frequency of the primary wave, and of the natural (*eigen*) frequency of the dipoles. If both frequencies are identical, resonance occurs and absorption results. The dispersion (spectral variation of the refractive index) within a given spectral region depends therefore on the location of the nearest absorption band, as shown in figure 10.¹⁷ The refractive index, n , increases rapidly as one approaches the band from longer wave lengths and varies anomalously with the wave length in the region of absorption (k = absorption coefficient). Cotton¹⁸ anticipated and found an analogous relation between absorption and the dispersion of n_d and n_u . The rotatory power increases very strongly as one approaches an appropriate absorption band and changes sign within the region of absorption

¹⁶ E. Fischer, *Ber.*, **24**, 2683 (1891); **27**, 3189 (1894).

¹⁷ For details on these and other elementary optical phenomena, see: G. S. Monk, *Light, Principles and Experiments*, McGraw-Hill, New York, 1937; R. W. Wood, *Physical Optics*, Macmillan, New York, 1934; J. Valasek, *Elements of Optics*, McGraw-Hill, New York, 1932.

¹⁸ A. Cotton. *Compt. rend.*, **120**, 989, 1044 (1895); *Ann. chim. phys.*, **8**, 347 (1896).

(Fig. 11). Not every absorption band, but only an "optically active" band, leads to anomalous dispersion of rotatory power. The latter is expressed in figure 11 in terms of $(n_d - n_l)$ (see page 1496). Light absorption within an optically active absorption band is different for the d - and l -component, an effect called circular dichroism, or Cotton effect. The magnitude of this effect is defined by $(k_d - k_l)$. It has become customary to include in the term "Cotton effect" the whole complex of phenomena illustrated by figure 11.

The circular dichroism changes linearly polarized light into elliptically polarized light (Fig. 19). A simultaneous rotatory power expresses itself by a noncoincidence of either axis of the ellipse with the direction of the initial linear vibration.

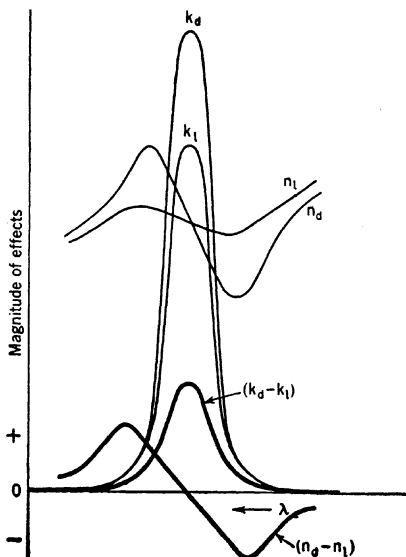


Fig. 11.—The Cotton effect.

As long as one is not interested in measuring the magnitude of circular dichroism and as long as the ellipticity remains fairly small, the measurement of rotatory power can be carried out in the usual manner. This is always possible with colorless liquids or solutions investigated in the visible region of the spectrum.

If the spectral location of the nearest optically active absorption band and the sign of the circular dichroism are known, the sign of α in the neighborhood of the band is given by Natanson's rule,¹⁹ which states that the less absorbed circular component is less refracted at larger wave lengths and more refracted at shorter wave lengths (see Fig. 11).

¹⁹ L. Natanson, *J. phys.*, 8, 321 (1909).

It follows from the preceding discussion that the determination of α for a single wave length is of limited significance, and that far more information on an optically active compound can be obtained by computing the dispersion of α (see page 1605).

3. Modern Theories

The discovery of the Cotton effect showed that, in any theory of rotatory power which is more than formal, the explanation of optically active absorption must be considered as the principal problem and the explanation of the circular double refraction as an intimately related problem.

In the first theory based on this concept,²⁰ Drude assumed that, in optically active compounds, electrons move in helical orbits. Drude's equations have a permanent value, although the concept of helical vibrators was abandoned.

The fundamental theory of Born²¹ can be summarized as follows:

(1) The harmonic linear resonators created in a molecule under the polarizing influence of an incoming electromagnetic wave, and leading to absorption, are not all mutually independent but may influence each other (coupling effect).

(2) For a very specific spatial arrangement of the coupled resonators—which satisfies Pasteur's principle of dissymmetry—an "optically active" absorption can be postulated. Both the circular dichroism and the related rotatory power are treated by the classical theory of dispersion.

A quantum mechanical treatment has been substituted for the classical treatment,²²⁻²⁷ and the theory has undergone several modifications and simplifications, making it possible to calculate numerically the rotatory power of molecules.^{28, 29-30} For instance, for diaminospiroheptane Born calculated a rotation, α , which differs only by 15% from the observed rotation.

Kuhn replaced Born's isotropic linear resonators, i. e., resonators with a frequency of vibration independent of the direction of vibration, by anisotropic linear resonators. While the resonators in Born's theory are electrons, the nature of Kuhn's anisotropic resonators is not definite. Nevertheless, Kuhn's theory proved very successful in explaining and predicting experimental regularities.

Kuhn's approach is based on the classical theory of dispersion. Condon²⁷ sub-

²⁰ P. Drude, *Lehrbuch der Optik*. 2nd ed., Hirzel, Leipzig, 1906.

²¹ M. Born, *Physik. Z.*, **16**, 251 (1915); *Ann. Physik*, **55**, 177 (1918). C. W. Oseen, *ibid.*, **48**, 1 (1915). See also A. Landé, *ibid.*, **56**, 225 (1918); R. Gans, *Z. Physik*, **17**, 353 (1923); *ibid.*, **27**, 164 (1924); *Ann. Physik*, **79**, 548 (1926).

²² L. Rosenfeld, *Z. Physik*, **52**, 161 (1928).

²³ M. Born and P. Jordan, *Elementare Quantenmechanik*. Springer, Berlin, 1930.

²⁴ G. Temple, *Trans. Faraday Soc.*, **26**, 293 (1930).

²⁵ P. P. Ewald, *Trans. Faraday Soc.*, **26**, 313 (1930).

²⁶ J. G. Kirkwood, *J. Chem. Phys.*, **5**, 479 (1937).

²⁷ E. V. Condon, *Rev. Modern Phys.*, **9**, 432 (1937).

²⁸ W. Kuhn, *Z. physik. Chem.*, **4**, 14 (1929); *Trans. Faraday Soc.*, **26**, 293 (1930).

²⁹ See particularly W. Kuhn and H. Freudenberg, "Natürliche Drehung der Polarisationsebene," in A. Eucken and K. L. Wolf, *Hand- und Jahrbuch der chemischen Physik*, Vol. 8, Part III, Akadem. Verlagsgesellschaft, Leipzig, 1936.

³⁰ M. Born, *Proc. Roy. Soc. London*, **A150**, 84 (1935).

stituted for it the quantum mechanical theory. Kuhn's elementary model of an optically active molecule, as shown in figure 12, consists of two anisotropic resonators, A and C, of unequal frequency which can vibrate in the direction of the arrows only, due to neighboring substituents B and D, respectively. An electric coupling force acts between A and C which leads, for example, to coresonance in C if the incoming light produces resonance in A. A "spread-out" absorption band results. If the directions of vibration of the resonators in A and in C are not in the same plane—they form an arbitrary angle of 90° in figure 12—circular dichroism and optical activity can be postulated. Depending on whether the resonators are in

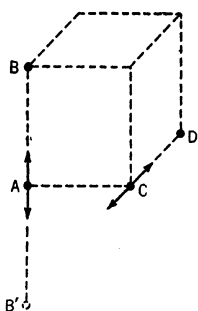


Fig. 12.—Kuhn's model of an optically active molecule.

phase or by 180° out of phase, the sign of the rotatory power will be positive or negative. The phase conditions are prescribed (a) by the sign of the coupling force, (b) by the frequency of the incoming light, (c) by the frequency relation of the resonators in A and C ($\nu_A > \nu_C$ or $\nu_A < \nu_C$), and (d) by the geometry of the structure. By keeping ACD constant, for example, B may be replaced by B' in an alternate model of the optical antipode. It is apparent from figure 12 that the simplest possible optically active molecule consists of two anisotropic resonators. It is dissymmetric in accordance with Pasteur's principle, and it contains four atoms or radicals, A, B, C, and D, arranged in a nonplanar structure, in accordance with the concept of van't Hoff. It is apparent also that a fifth atom—e. g.,

a carbon atom—is of no importance for the production of optical activity. Its presence is required for chemical reasons only. One of the most important predictions made by Kuhn is that the rotatory power should disappear both in the region of long Hertzian waves and in the region of x-rays and should be strongest in the ultraviolet.^{29, 31} It seems to be borne out by data available on quartz. A 1-mm. layer produces a rotation of 0.52° , 1.63° , 6.18° , 21.75° , 370.9° , and 3.33° at 32,100, 20,000, 10,800, 5890–5896, 1854, and $\sim 20 \text{ \AA}$., respectively. The last value was obtained with a linearly polarized beam of x-rays traversing a crystal parallel to its optic axis.³²

It may be mentioned that some useful theories do not attempt an explanation of optical activity on the basis of the elementary physical processes in a molecule. Instead, they derive the rotatory power from nonelementary properties, e. g., from the refractivity of the various atoms or atom groups of the molecule. It is possible to calculate by such methods³³ the approximate rotatory power of simple

²⁹ J. P. Mathieu, *Compt. rend.*, **214**, 420 (1942).

³⁰ R. L. MacFarlan, *Phys. Rev.*, **45**, 292 (1934).

³¹ E. g., P. de Malleman, *Ann. phys.*, **2**, 5 (1924); **4**, 456 (1925); *Compt. rend.*, **181**, 298 (1925); **184**, 1241, 1374 (1927); **185**, 350 (1927); **186**, 1046 (1928); **188**, 705 (1930); *Trans. Faraday Soc.*, **26**, 281 (1930).

molecules, particularly when taking into account the mutual interaction of the individual groups.³⁴

Attention may also be drawn to the so-called "one-electron theory,"³⁵⁻³⁸ which is theoretically interesting, but cannot give as yet more than the order of magnitude of the effects to be expected from a molecule.

There is, finally, a large number of "rules" of empirical origin by means of which correlations are established between the structure of a molecule and its rotatory power.³⁹

IV. DISTINCTION BETWEEN NATURAL ROTATORY POWER AND OTHER PERTINENT PHENOMENA OF ROTATION

1. Linear Dichroism

An optically active substance rotates the direction of vibration of linearly polarized light. Rotation observed during the passage of a linearly polarized beam through a liquid or a solution is, however, not necessarily a proof of optical activity, a fact which is often overlooked and which deserves some attention. Rotation may also be caused by linear dichroism. This, the linear analogue to circular dichroism, is the effect of an anisotropic absorption in linearly anisotropic substances. The elementary optics of such substances is presented on pp. 1508 *et seq.* In figure 13, V_0 is considered the resultant of two coherent orthogonal linear components, V_{0x} and V_{0y} . This is similar to the decomposition of a linear vibration into two coherent circular components of opposite sense of rotation (Figs. 3, 5) in the case of circularly anisotropic substances. The absorption coefficients, k_x and k_y , of the dichroitic substance are unequal, so that the amplitudes, a_{0x} and a_{0y} , of the two components are reduced to a_{1x} and a_{1y} , respectively, during the passage of the beam through the substance. The recombination of the two components leads then to a re-

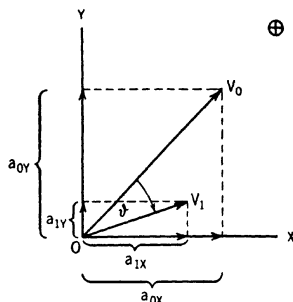


Fig. 13.—Optical rotation due to linear dichroism.

³⁴ See particularly S. F. Boys, *Proc. Roy. Soc. London*, **A144**, 655 (1934). See also F. Gray, *Phys. Rev.*, **7**, 472 (1916).

³⁵ E. V. Condon, W. Altar, and H. Eyring, *J. Chem. Phys.*, **5**, 753 (1937).

³⁶ E. Gorin, W. J. Kauzmann, and J. E. Walter, *J. Chem. Phys.*, **7**, 327 (1939).

³⁷ E. Gorin, J. E. Walter, and H. Eyring, *J. Chem. Phys.*, **6**, 824 (1938).

³⁸ W. J. Kauzmann, J. E. Walter, and H. Eyring, *Chem. Revs.*, **26**, 339 (1940).

³⁹ See, for example, R. E. Marker, *J. Am. Chem. Soc.*, **58**, 976 (1938).

sultant linear vibration, V_1 , which is rotated, by the angle ϑ , with respect to V_0 . In figure 13, an angle V_0OX of 45° is assumed. If the angle is either smaller or larger, the rotation is smaller. It would be zero for an angle of 0° or 90° . This gives us the criterion for distinguishing between a rotation due to natural rotatory power and a rotation due to linear dichroism: angle α is independent of the direction of vibration of the incident beam, while angle ϑ varies with this direction. The safest practical method of distinguishing between the two effects is the rotation of both polarizer and analyzer, in crossed position, in the same sense and to the same extent. The light transmission will remain unaltered in the case of rotatory power. However, if linear dichroism causes the rotation, then rotating the polarizer and analyzer will lead to changes in the brightness of the field. In the case of the extinction method (Fig. 29 and page 1534), for example, the transmission changes between zero and a maximum, twice during a complete rotation of polarizer and analyzer, by 360° . Linear dichroism is unlikely, in the absence of an external orienting factor, unless the anisotropic substance represents a structured colloid in which the spontaneously oriented elements are anisometric.⁴⁰ One can predict that the effect will occur, for example, in protein structures, particularly in the ultraviolet. Such structures may also be optically active. A linear dichroism is particularly likely, however, if linearly dichroic molecules or particles are oriented by an external force. The danger of confusing linear dichroism with rotatory power is therefore particularly high in a transverse electric or magnetic field, or in streaming solutions.^{41,42} A linear dichroism may not only be due to anisotropic true absorption, but also to anisotropic light scattering.⁴³ The color of the substance is therefore no reliable criterion for the presence or absence of dichroism.

2. Faraday Effect

Parallel to the lines of force of a magnetic field, a "magnetic rotatory power" known as the Faraday effect is observed in any liquid or solution.⁴⁴ Just as rotatory power in a spectral region close to an optically active absorption band is accompanied by circular dichroism, the Faraday effect is accompanied by a magnetic circular dichroism (inverse Zeeman effect) under similar conditions. The Faraday effect, like the related Zeeman

⁴⁰ W. Heller, in R. Abegg, *Handbuch der anorganischen Chemie*. Vol. 4, Hirzel, Leipzig, 1935, Part III, 2B, p. 860.

⁴¹ J. Kunz and R. G. LaBaw, *Nature*, **140**, 194 (1937).

⁴² E. B. Ludlam, A. W. Pryde, and H. Gordon-Rule, *Nature*, **140**, 194 (1937).

⁴³ W. Heller, *Rev. Modern Phys.*, **14**, 406 (1942).

⁴⁴ M. Faraday, *Phil. Mag.*, **28**, 294; **29**, 153 (1846).

effect,⁴⁵ results from an influence of the magnetic field upon the electron movement within the atoms, and the sign of the effect depends on the direction of the magnetizing current. In most instances, the rotation, χ , has the same direction as the magnetizing current, as illustrated by figure 14. The effect is then called positive. It follows from figure 14 that the dependence on the direction of the current leads to two characteristic properties of the Faraday effect: (1) If the beam is reflected by a mirror, M , and returns through the substance, the effect observed at O' will be double since the direction of the current is the same both times. (2) To the observer at O , in the absence of M , the rotation appears as positive, which it is according to the definition given above. However, if we interchange the light source, S , and the position of the observer at O , the rotation appears negative, although it is still positive according to the definition. Consequently, the apparent, not the true, sign of the Faraday

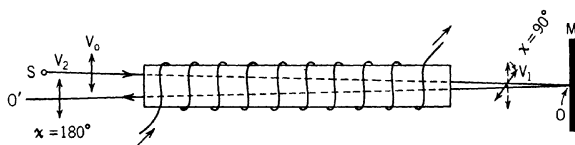


Fig. 14.—The Faraday effect.

effect depends on the direction of observation, while the magnitude of the effect is independent of it. The situation is different in the case of natural rotatory power. Here, the sign of the rotation is independent of an interchange between light source and observer. It follows implicitly that the result of reflection is different here. The rotation due to natural rotatory power is annulled if the light beam is reflected and thus forced to travel twice through the substance, in opposite directions.

An optically active substance observed in a longitudinal magnetic field will therefore exhibit two types of rotation, a natural rotatory power superimposed upon which is a magnetic rotatory power. The two effects can be separated indirectly (they may have opposite signs) by carrying out an experiment, first in zero field and then in a finite field. Whether the field exerts an influence upon the natural rotatory power itself is, to the knowledge of the author, still an open question. It could be solved by a third experiment, in which the beam traverses the substance in the magnetic field two or $2n$ times in opposite directions. Organic substances exhibiting a well measurable Faraday effect are much more numerous than those having natural rotatory power. However, the investigation of the Faraday

⁴⁵ P. Zeeman, *Proc. Acad. Sci. Amsterdam*, 5, 41 (1902).

effect has, by comparison, been neglected. Table I gives the Faraday effect of a few selected organic liquids.⁴⁶

TABLE I
FARADAY EFFECT OF SELECTED ORGANIC LIQUIDS

Substance	Wave length, Å.	Temperature, ° C.	χ^a
Methanol	5893 \pm 3	19	1° 39'
Ethanol	4529	16	3° 15'
Methyl chloride	5893 \pm 3	18	2° 9'
Carbon tetrachloride	5893 \pm 3	15	2° 40'
Carbon disulfide	5893 \pm 3	18	7° 10'
Acetone	5893 \pm 3	15.1	1° 51'
Sucrose	4860	20	1° 43'
Benzene	5893 \pm 3	23.8	4° 48'
Nitrobenzene	5893 \pm 3	22.0	3° 36'
α -Bromonaphthalene	5893 \pm 3	24.1	8° 21'

^a Rotation χ calculated for a layer of 1 cm. in longitudinal magnetic field of 10,000 gauss.

The polarimetric technique for investigations of the Faraday effect is identical with that to be described for the natural rotatory power, so that a special treatment is unnecessary. In a number of instances, particularly in photoelectric polarimetry (pages 1589 *et seq.*), simpler methods are usable for measuring the Faraday effect than the natural rotatory power. Such cases will be discussed (page 1598).

V. TECHNIQUE OF POLARIMETRIC MEASUREMENTS

Polarimetric measurements of rotatory power are concerned with the direct or indirect determination of the angle of rotation, α . While this operation appears simple, any polarimeter for precision measurements applies quite a number of optical phenomena. The chemist interested in precision measurements is therefore likely to encounter difficulties if he is not familiar with the functioning of the various parts of a polarimeter; to give this information is the first aim of the present chapter. A number of excellent polarimeters are on the market, but many problems require the construction of special equipment; to furnish the basic knowledge necessary for such a task is the second aim of the present chapter.

1. Introduction to Elementary Optics of Linearly Anisotropic Substances

We have seen that optically active substances respond differently to a right and a left circular vibration (circular anisotropy). There is a second, much larger,

⁴⁶ For a comprehensive treatment of the Faraday effect, see R. Ladenburg, in J. Müller and C. Pouillet, *Lehrbuch der Physik*. 11th ed., Vol. 2, Vieweg, Braunschweig, 1929, Part II, pp. 2119-2179.

class of substances which respond differently to two orthogonal linear vibrations (linear anisotropy). Such substances are of paramount importance in polarimetric technique, where extensive use is made of the phenomenon of linear double refraction.

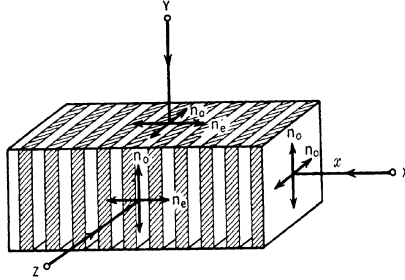


Fig. 15.—Double refraction of a morphically anisotropic substance.

tion. This effect, and some resulting phenomena, will be outlined below, to the extent required for the discussion of polarimetric measurements of rotatory power.

The cause and the optical consequences of linear double refraction may be visualized most easily on the example of "morphically" double refracting (bire-

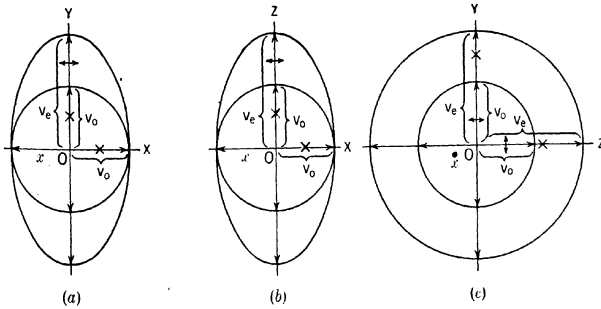


Fig. 16.—Wave surfaces of uniaxial negative substance ($n_e < n_o$) if light emanates from center of substance.

a , b , and c are cross sections through the three-dimensional scheme of wave surfaces. The ellipse is exaggerated for the sake of demonstration; it actually is almost a circle. v represents light path per unit time (velocity).

fringent) substances. Figure 15 represents a rectangular rod which consists of a large number of isotropic solid sheets separated from each other by a gas or liquid. Both the thickness of the sheets and their mutual distance are small compared

with the wave length of light. According to Wiener,⁴⁷ such a rod will exhibit linear double refraction. If light is propagated along X , i. e., perpendicular to the sheets, the rate of propagation will be the same for a vibration $\parallel Y$ and for a vibration $\parallel Z$. If light is propagated along Y or Z , however, the rate of propagation will vary with the direction of vibration. If the direction of vibration is $\perp X$, the rate will be equal to that found in the case of propagation along X . It will be smaller if the direction of vibration is parallel to X . Since the rate of propagation of a linearly polarized beam depends on the refractive index in the direction of vibration, the stratified rod can be characterized by two different refractive indices, one, n_e , for vibrations parallel to X and another, n_o , for vibrations perpendicular to X . The X direction, a privileged direction, is called the "optic axis," x . The principal

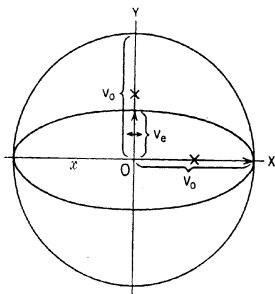


Fig. 17.—Wave surfaces of uniaxial positive substance ($n_e > n_o$). As in figure 16, the ellipse is much closer to being a circle than the figure shows. For V , see Fig. 16.

"extraordinary index" is n_e , and n_o is the "ordinary index." If $n_e > n_o$, the birefringent substance is called "uniaxial positive" and the double refraction is called positive because $(n_e - n_o)$ is positive. If $n_e < n_o$, the substance is called "uniaxial negative," and the double refraction is called negative. The term "uniaxial" indicates that the substance has one optic axis. A linearly anisotropic uniaxial substance is optically characterized by the direction of the optic axis, the ordinary refractive index, and the principal extraordinary refractive index. Substances which have two optic axes, i. e., three different refractive indices, are not of interest here.

The distance covered, in a certain very small period of time, by a light impulse created in the origin, is plotted in figure 16.

The origin is assumed to be in the interior of an anisotropic substance. Only a propagation parallel or perpendicular to the optic axis will be considered. Light propagated along the optic axis, which coincides in the figure with X , can vibrate only perpendicular to X . Consequently there will be only one rate of propagation in the X direction (ordinary velocity, v_o , determined by n_o). Light propagated in the Y or Z direction may vibrate either parallel or perpendicular to the optic axis. Consequently the light propagated in these directions will consist of two components, one propagated with the ordinary velocity and the other propagated with a "principal extraordinary" velocity, v_e . It follows that any point reached after a certain time by the light impulse lies on one or the other of two wave surfaces. One of the surfaces is that of a sphere, i. e., identical with the wave surface of an isotropic substance of refractive index n_o . The other surface is that of an ellipsoid. The latter is obviously tangent to the former at the points of intersection with the optic axis, which is the symmetry axis of revolution of the ellipsoid. In figure 16 $v_e > v_o$; in figure 17 $v_e < v_o$.

⁴⁷ O. Wiener, *Abhandl. math.-phys. Klasse sächs. Akad. Wiss. Leipzig*, 32, 509 (1912).

We now study the propagation of a pencil of parallel natural light incident upon a uniaxial negative crystal. There are three possibilities of orientation for the optic axis with respect to the crystal face through which the beam enters: the axis and the face may form an angle of 90° , of 0° , or of $\neq 90^\circ \neq 0^\circ$. The direction and the rate of propagation of light in the crystal shall be established for all three cases.

By restricting the discussion to perpendicular incidence, which is sufficient for our needs, it can be postulated that the light travels in that direction in which it penetrates deepest into the crystal within a certain time, taking the distance from the face as the criterion. The deepest penetration and the direction of propagation are then defined by the point at which a plane parallel to the face of the crystal is tangent to the wave surface (Fig. 18). The double-headed arrow gives the direction

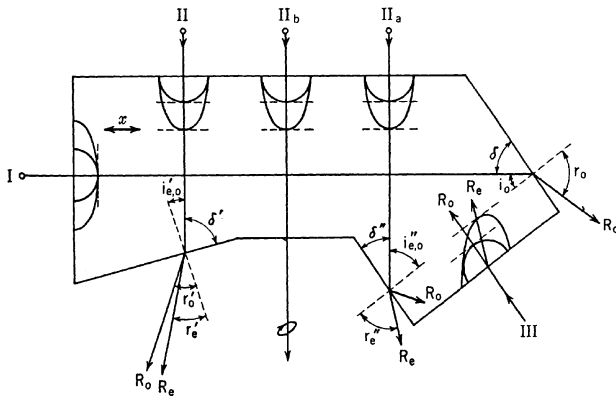


Fig. 18.—Refraction, reflection, and interference phenomena of a light beam traversing a uniaxial crystal.

of the optic axis. If the optic axis is perpendicular to the face of the crystal (I), the direction of propagation leading to deepest penetration is in the direction of the optic axis. The light beam is therefore propagated without deviation and at the rate v_o , i. e., the beam is propagated as in an isotropic substance. If the optic axis is parallel to the face of the crystal (II), the light will again travel through the crystal without deviation, but this time there will be two components. They travel along the same path, but at different rates, v_o and v_e . If the optic axis forms an angle $\neq 0^\circ$ and $\neq 90^\circ$ with the face of the crystal (III), the beam will be split up into two components, one propagated without deviation at the rate v_o , the other propagated under deviation at the rate v_e . The latter component is called the "extraordinary" ray, R_e , and the former, the "ordinary" ray, R_o . The direction of vibration of the ordinary ray is perpendicular to the optic axis; the direction of vibration of the extraordinary ray is in a plane parallel to the optic axis.

The next problem of importance is that of refraction and reflection, respectively,

at the point at which the pencil of light reaches the end of the crystal. According to Snell's law of refraction (see page 1142, Chapter XX):

$$n_e \sin i = n_1 \sin r_e; \quad n_o \sin i = n_1 \sin r_o \quad (7)$$

$$\therefore \sin r_o / \sin r_e = n_o / n_e \quad (8)$$

where n_1 is the refractive index of air, and i and r are the angles of incidence and refraction, respectively. In figure 18 I, the beam is refracted by angle r_o . In case II, it is split up into two divergent rays, the ordinary and the extraordinary. As

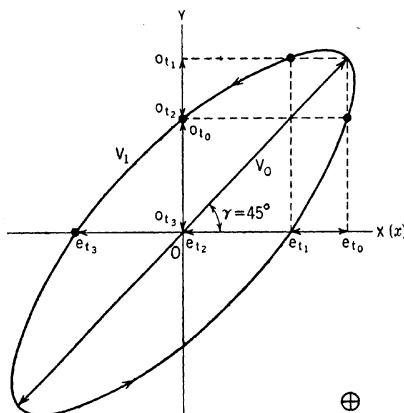


Fig. 19.—Elliptical polarization (Case IIb of Fig. 18).

regards reflection back into the crystal, the critical angle of incidence leading to total reflection, i_{cr} (page 1184) is reached if:

$$\sin i_{e_{cr}} = n_1/n_e; \quad \sin i_{o_{cr}} = n_1/n_o \quad (9)$$

$$\therefore \sin i_{e_{cr}} / \sin i_{o_{cr}} = n_o/n_e \quad (10)$$

The gradual reduction in angle δ (Fig. 18) leads therefore to three characteristic situations in the case considered: (a) two divergent linearly polarized components emerge (II); (b) only one linearly polarized component emerges, the second being totally reflected (IIa); (c) both components are totally reflected, a case not shown in the figure. The case $\delta = 90^\circ$, i. e., a plane-parallel crystal plate (IIb), is discussed below.

If the incident light is not natural, but is linearly polarized the phenomena discussed thus far by means of figure 18 remain unchanged but the physical relationship of the ordinary and extraordinary components becomes different. When natural light is split into two orthogonal linearly polarized components, these

components are incoherent, just as two circular components are incoherent if they are obtained from natural light. However, if a linearly polarized beam is split into two orthogonal linearly polarized components, one vibrating parallel to the optic axis, the other vibrating perpendicular to it (Fig. 19), the components are coherent, just as two circular components are coherent if they are obtained from linearly polarized light. This difference leads to a characteristic difference in the behavior of an originally natural and of an originally linearly polarized beam if $\delta = 90^\circ$ (Fig. 18, IIb). In the former case, the two components, traveling along the same path, behave in every respect as natural light. However, if the original beam is linearly polarized, then the coherent orthogonal components will interfere. The resultant beam is therefore generally elliptically polarized, and only under very particular conditions will it be linearly or circularly polarized. This is explained by figure 19, in which it is assumed that the passage through the plane-parallel uniaxial negative crystal plate has led to a path difference of $\frac{1}{8}$ wave length (phase difference of -45°). No discussion of the figure appears to be necessary, since it is drawn according to the same principle as figures 4 and 5. Figure 20 gives the variation in type and state of vibration with the phase difference if $\gamma = 45^\circ$. If $\gamma < 45^\circ$, the picture does not change characteristically except for phase differences of -90° and -270° . For these, elliptical vibrations with the long axis parallel to x are obtained instead of circular vibrations, as demonstrated in one case by a broken curve. If $\gamma = 0$ or $= 90^\circ$, the linearly polarized beam remains obviously unaltered. The types of vibration resulting from the various phase differences are the same, as above, if the optic axis coincides with the Y axis, or if the crystal is uniaxial positive with the optic axis parallel to the X axis. The sense of rotation of the elliptical and circular vibrations is then reversed, however.

Figure 20 suggests the procedure in measurements of the Cotton effect if one is interested in obtaining the absolute values of k_d and k_l by means of a photometer. A birefringent

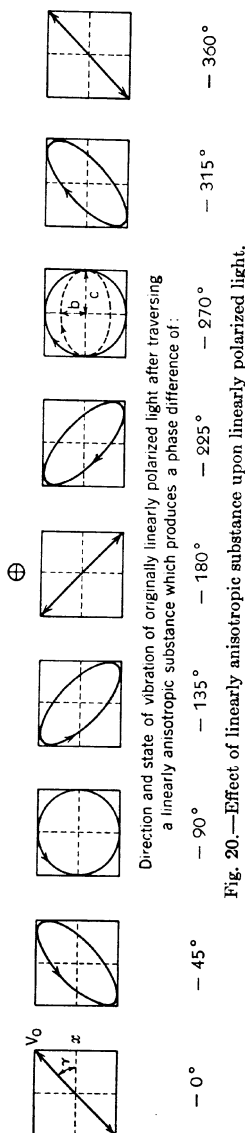


plate with a phase difference of $\pm 90^\circ$, under an angle, γ , of 45° , produces left circular or right circular light, respectively, from linearly polarized light depending on whether its optic axis coincides with the X axis or Y axis. Because of the dispersion of double refraction, measurements with a single plate are restricted to one wave length. Far better in this respect are Fresnel rhombs.⁴⁸ There are several other possibilities for measuring the Cotton effect, particularly the difference, $(k_d - k_l)$, which are not discussed here because they are beyond the scope of this chapter.⁴⁹

2. Basic Parts of a Polarimeter

A. POLARIZER

The polarizer produces linearly polarized light from natural light.

Polarizers Based on Double Refraction.—Cases *II*, *IIa*, and *III* of figure 18 lead to the construction principles of the most important types of polarizers. Most frequently used are those polarizers which do not affect the direction of propagation of the light. In these, corresponding to case *IIa*, one component of the light is totally reflected. Deviation of the transmitted component is prevented by combining two equally cut half-prisms of a uniaxial crystal. We

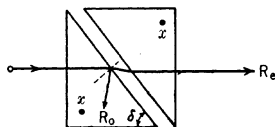


Fig. 21.—Glan prism.

shall first discuss the working conditions of prisms if used with parallel light and with an angle of incidence of 0° . In the Glan prism,⁵⁰ the two half-prisms are separated by a thin layer of air. Figure 21 exaggerates the thickness of this layer and the parallel displacement of the emerging beam in order to show the principle. The totally reflected ordinary ray is absorbed by black paint on the lateral face of the double prism.

The Glan prism and practically all other polarizing prisms based on total reflection are made of calcite, a uniaxial negative crystal with particularly strong double refraction (n_o , 1.4865; n_e , 1.6584; D line). Calcite is soft, and any scratch will seriously impair the polarizing quality of a prism by the scattering of light. In cleaning the faces of calcite polarizers, only lens paper or chamois skin should be used. Loose dust should be brushed off with a very soft camel's hair brush. In recent years, some progress has

⁴⁸ A. Fresnel, *Ann. chim. phys.*, 28, 147 (1825); W. Kuhn and E. Braun, *Z. physik. Chem.*, B7, 292; *ibid.*, B8, 445 (1930).

⁴⁹ For further information, see S. Mitchell, *The Cotton Effect and Related Phenomena*. Bell, London, 1933.

⁵⁰ P. Glan, *Ann. Physik Chem.*, 1, 351 (1877); *Repertorium Exptl.-Physik. (Carl's)*, 16, 570 (1880); 17, 195 (1881).

been made in growing large, optically faultless, and even more strongly double refracting crystals of sodium nitrate⁵¹ (n_e , 1.336; n_o , 1.587; D line). Polarizing prisms of this material can be made much shorter than calcite polarizing prisms with the same field and aperture, as will follow from a discussion of this point on pp. 1517 *et seq.* They are thus of very definite interest, notwithstanding (a) their solubility in water and (b) the difficulties involved in finding a cementing substance of sufficiently low refractive index, $n \sim n_e$.

The beam emerging from a Glan prism is slightly displaced (but parallel) with respect to the incident beam. Rotation of the prism around the direction of light propagation as the axis causes the polarized beam to wander along a circle. This imperfection may be negligible if the air interlayer is very thin. More important is the twofold reflection of the trans-

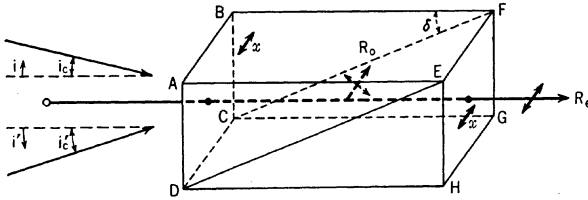


Fig. 22.—Glan-Thompson prism.

mitted polarized ray at the air layer, which results in a loss of polarized light. The main deficiency is the small field of the Glan prism—a deficiency existing for all polarizers with air interlayer. This means that perfect polarization can be obtained only if the incident beam represents parallel light and if the angle of incidence is 0° . The permissible maximum deviation from this condition is $\pm 4^\circ$ for the Glan (see also the discussion on pp. 1517 *et seq.*).

Both disadvantages are eliminated in the Glazebrook or Glan-Thompson polarizing prism⁵² (Fig. 22). Here, the two half-prisms are cemented with Canada balsam. The refractive index of the latter (1.55) is so close to n_e of calcite (1.49) that the loss of intensity of the transmitted extraordinary ray is much smaller than with the Glan prism. On the other hand, n of Canada balsam is also much closer to n_o of calcite (1.66) than is n of air. According to equation (9), δ , which is $(90^\circ - i)$ here, must therefore be reduced in order to maintain total reflection of the ordinary ray. Conse-

⁵¹ See, for example, B. K. Johnson, *Proc. Phys. Soc. London*, 55, 291 (1943).

⁵² R. T. Glazebrook, *Phil. Mag.*, 10, 247 (1880); 15, 352 (1883). S. P. Thompson, *ibid.*, 12, 349 (1881); 15, 435 (1883); 21, 476 (1866).

quently, the ratio of length to width is much larger for Glan-Thompson prisms than for Glan prisms of equal width. The internal reflection of the extraordinary ray can be completely avoided by using linseed oil ($n = 1.486$) between the two half-prisms. With this intermediate, the prisms can be approximately 10% shorter than prisms cemented with Canada balsam. Optical companies now use resins which combine cementing qualities with a refractive index close to that of linseed oil. The optic axis, parallel to the ground and polished face of the crystal, through which the light enters, is parallel to AB . It may be parallel to AD instead, which, however, is slightly less advantageous (Ahrens prism).⁵³ In some instances, the axis is parallel to AC or BD . The optic axis in calcite crystals is strongly oblique with respect to any of the natural faces. A crystal must therefore

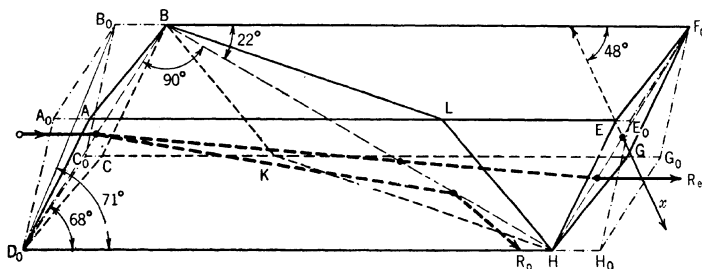


Fig. 23.—Nicol prism with angles and other dimensions drawn out of proportion to show principles of construction.

$A_0B_0C_0D_0$ and $E_0F_0G_0H_0$, faces of original calcite crystal; $ABCD_0$ and EF_0GH_0 , faces of crystal after correction; $BLHK$, plane along which corrected crystal is cut and recemented.

be cut down considerably for a Glan-Thompson prism, which consequently is expensive.

The nicol prism,⁵⁴ shown in figure 23, is much cheaper. The faces of the natural crystal are less corrected, leaving the optic axis oblique with respect to the frontal face, $ABCD_0$. In addition, this face is oblique to the lateral faces, and the angle of incidence of the light beam is $\neq 0^\circ$. Two characteristic properties are the result: the beam is split into two components immediately upon entering the nicol prism; and both the extraordinary and the ordinary ray are deflected.

The nicol prism has two disadvantages which exclude its use in precision measurements: (1) The light beam emerging from the polarizer moves in

⁵³ Constructed by C. D. Ahrens. See S. P. Thompson, *Phil. Mag.*, 21, 476 (1886).

⁵⁴ W. Nicol, *Edinburgh New Phil. J.*, 6, 83 (1828); 16, 372 (1834).

a circle as the polarizer is rotated, a defect evident from figure 23, and much stronger than with the Glan prism (Fig. 21, p. 1514). (2) If used as an analyzer, in combination with a polarizing nicol and a very bright light source (see page 1527), complete extinction cannot be brought about for the whole optical field, but only for a limited section of it, and the locus of this section within the field varies with the rotation of the analyzer around the true position of "crossed nicols." The meaning of statement (2) may become fully clear only after the discussion of the polarizer-analyzer combination (pp. 1524 *et seq.*). According to Bruhat, the latter deficiency of the nicol prism introduces an uncertainty of several minutes in the determination of the angle of rotation.⁵⁵ If used in a polarizing microscope, the nicol prism introduces astigmatism, a third deficiency, which is unimportant, however, at low magnifications, and irrelevant in those polarimetric methods not involving the use of a microscope.

It has become customary to call calcite polarizers and analyzers of any type "nicols," provided they are composed of two half-prisms with equally oriented optic axes. We shall use the term in this sense.

Under actual working conditions the light incident upon a Glan-Thompson prism may be slightly convergent or divergent, so that the angle of incidence is 0° for a fraction of the beam only. However, only a limited deviation from 0° is permissible. This may be discussed by means of figure 22. We assume that the two half-prisms are separated by air and vary the angle of incidence in a plane perpendicular to $ABCD$ and parallel to AD (BC). For an angle of incidence, i_c , both the extraordinary and ordinary component are transmitted; if the angle of incidence is i'_c , both components are totally reflected at the diagonal plane inside the prism. For intermediate angles, the extraordinary component is transmitted, and the ordinary component is totally reflected. The critical angle—which cannot be exceeded if polarization is to be complete—is characterized by the subscript c . The sum ($i_c + i'_c$) defines the permissible deviation from normal incidence. Actually, the two half-prisms are not separated by air, but by Canada balsam. The angle i'_c is then obviously far larger, another important advantage of Glan-Thompson prisms over Glan prisms. If the refractive index of the cement equals n_o , then i' has no significance, since total reflection of the extraordinary component is impossible. The "field" of the polarizing prism, *i. e.*, the permissible solid angle of the light cone, then depends exclusively on i_c which increases with decreasing δ and therefore with increasing ratio of length to width (AE/EH) of the prism. i_c is calculated on the basis of equations (7) and (9). For example, angle $2i$, defining the cone of a convergent beam, must not exceed 4° if the ratio

⁵⁵ G. Bruhat and M. Hanot, *J. phys.*, 3, 46 (1922).

AE/EH is 2.15. If the prism were cemented with Canada balsam, the permissible angle $2i_c$ would be smaller; and, in order to make it 4° , the ratio AE/EH would have to be 2.81.

The most important practical conclusions are the following: (a) The incident beam must not be more convergent or divergent than is compatible with the polarizer field; otherwise, partially natural light will be transmitted. It is therefore wrong to concentrate a beam upon the polarizer in the futile hope of getting "more intensity" in the apparatus. (b) If one

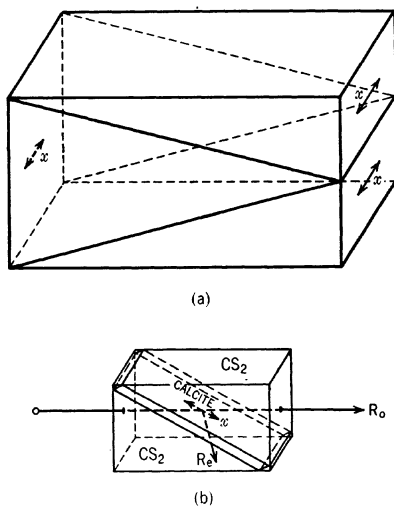


Fig. 24.—Calcite polarizers with large aperture. (a) Ahrens-Bénard prism; (b) Jamin-Brace "sensitive strip" polarizer.

receivements a polarizing prism with Canada balsam—which must be free from dust in order to exclude depolarizing light scattering— i_c will be smaller than indicated by the manufacturer if the prism was cemented with a resin of refractive index closer to n_c . (c) The polarizer must be lined up properly. Even with a beam of parallel light, one will not obtain completely polarized light if the angle of incidence is $> i_c$. In order to test the proper alignment of the polarizer, of the light source, or of the condensing lens in front of the light source, a sheet of stiff black paper with a small hole is placed between condensing lens and polarizer. The light reflected from the face of the polarizer must be centered at the hole and must remain there upon rotation of the polarizer.

The aperture, $ABCD$, of a polarizer is determined by its length, since the ratio AE/EH is constant for constant i_e , and consequently large apertures require long polarizers. If, for example, one wants to increase the aperture of a square prism from 1 to 2 cm.², while maintaining the same i_e , then the length of the prism must be doubled. Glan-Thompson polarizers with larger apertures are therefore very costly; if a large aperture is required, an Ahrens-Bénard⁵⁶ prism (Fig. 24a) may be used. The square prism has the aperture of a square Glan-Thompson prism twice as long; therefore, a much smaller calcite crystal can be used for its construction. Light scattering by the apex of the central wedge, if Canada balsam is used, produces interference fringes which may disturb the homogeneity of the optical field in bright illumination. Nevertheless, the prism is much used in polarizing microscopes. A theoretically better solution is the "sensitive strip" polarizer of Jamin⁵⁷ (Fig. 24b), in which the construction of a Glan-Thompson prism is inverted. The two half-prisms are replaced by carbon disulfide and the layer of Canada balsam is replaced by a strip of calcite. Under these conditions, the ordinary ray is transmitted. Brace⁵⁸ substituted monobromonaphthalene for carbon disulfide. This type of polarizer can be constructed at a comparatively low cost considering the large aperture obtained. A disadvantage is the absolute necessity of having a perfectly dust-free liquid; otherwise, only a partial polarization can be achieved.

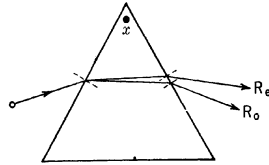


Fig. 25.—Double image polarizing and dispersing prism.

For certain purposes (page 1580), polarizers consisting of a single prism of calcite, simple "double image" prisms, are preferable. Polarization is then accompanied by a deflection of either polarized component. The most suitable type is shown in figure 25. Generally, the more refracted component is used. The interesting feature of these polarizers is their simultaneous action as a dispersing prism (page 1581).

When both the ordinary and the extraordinary components are to be used (page 1593), two types of polarizers are particularly practical: (a) A single birefringent crystal, like calcite, under normal incidence (Fig. 26a) transmits the ordinary ray without deviation. The extraordinary ray is displaced laterally, but runs parallel to the ordinary ray after leaving the crystal. The calcite crystal should be three inches long in order to give

⁵⁶ H. Bénard, *Recherches inventions*, 1, 229 (1920).

⁵⁷ J. Jamin, *Compt. rend.*, 68, 221 (1869).

⁵⁸ D. B. Brace, *Phil. Mag.*, 5, 161 (1903).

satisfactory separation of ordinary and extraordinary rays.⁶⁰ (b) A composite "double image" prism of calcite, *e. g.*, a Wollaston prism⁶⁰ (Fig. 26b) displaces ordinary and extraordinary rays nearly symmetrically from the original direction of propagation. Closely related to the Wollaston prism are the Rochon prism⁶¹ and the Senarmont prism,⁶² in which one of the components is not deviated. In order to increase the angular separation of the two components, double image prisms have been constructed with three prisms (Abbe prism)⁶³ or even four individual prisms (Ahrens prism),⁶⁴ one or two of the latter consisting of glass.

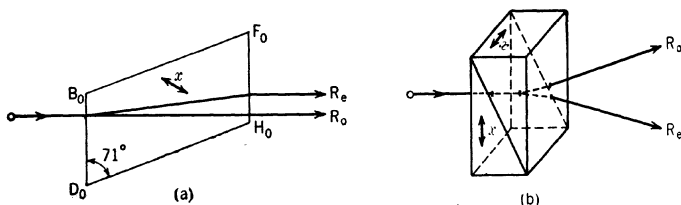


Fig. 26.—Double image polarizing prisms: (a) natural calcite crystal (for the meaning of $B_0D_0F_0H_0$, see figure 23); (b) Wollaston prism.

Polarizers Based on Reflection.—The use of calcite and other birefringent materials is limited to spectral regions where these substances do not absorb light strongly. In regions of strong absorption (see pages 1575 and 1577), polarization by reflection is used. According to Brewster's law, a beam reflected on a mirror ($ABCD$, Fig. 27) contains only one component, that vibrating perpendicular to the plane of incidence, SFF' (view I), if:

$$\tan i = n_M \quad (11)$$

where n_M is the refractive index of the reflecting mirror. Table II gives Brewster's angle for quartz, several kinds of glass, and plastics. An advantage of these polarizers is that the width of the polarized beam is not as limited as in the case of polarizing prisms. A major disadvantage, however, is the relatively low intensity of polarized light that can be obtained with such a polarizing mirror (see page 1526).

While Brewster's law defines the angle of incidence required for complete polarization by reflection, partial polarization occurs under all other angles

⁶⁰ W. Heller and G. Quimfe, *Phys. Rev.*, **51**, 382 (1942).

⁶¹ W. H. Wollaston, *Trans. Roy. Soc. London*, **A110**, 126 (1820).

⁶² A. Rochon, *J. phys.*, **72**, 319 (1811).

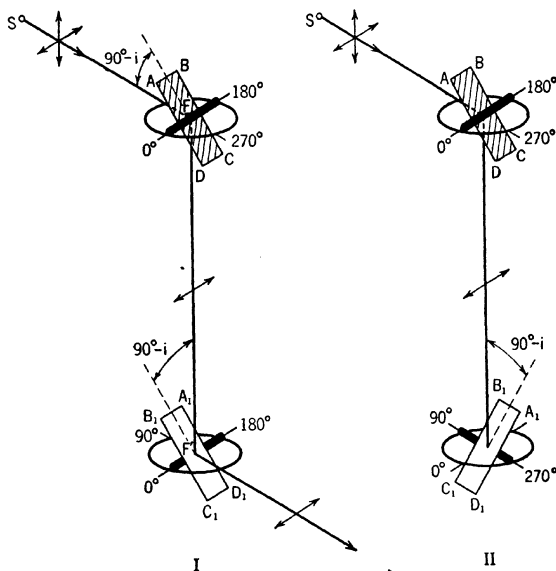
⁶³ H. de Senarmont, *Ann. chim. phys.*, **50**, 480 (1857).

⁶⁴ See W. Grosse, *Thesis*, Univ. of Kiel, 1886.

⁶⁵ C. D. Ahrens, *Phil. Mag.*, **19**, 69 (1885).

TABLE II
BREWSTER'S ANGLE, i , FOR VARIOUS REFLECTORS

Substance	Refractive index (5890-5896 Å.)	δ
Fused quartz	1.458	55° 33'
Lucite	1.50 \pm 0.01	56° 19' \pm 11'
Light crown glass	1.517	56° 36'
Crown glass	1.520	56° 40'
Flint glass	1.575	57° 35'
Polystyrene	1.595 \pm 0.003	57° 55' \pm 3'
Heavy flint glass	1.650	58° 47'
Very heavy flint glass	1.890	62° 7'



vibration of the polarized fraction of light emerging from a monochromator is perpendicular to the length of the exit slit. The degree of polarization increases, of course, with the number of reflections. Thus, 31% of the light emerging from a double monochromator with glass prisms is linearly polarized. In order to take full advantage of the brightness of the light source, it is imperative to arrange the polarizer so that its direction of transmission is crossed with respect to the exit slit of the monochromator. The technique used for identifying the direction of transmission is described on page 1525.

Polarizers Based on Dichroism.—A third principle of polarization may be applied at certain wave lengths if no precision is required in the measurements. Not only the refractive indices, but also the absorption coefficients are different parallel and perpendicular to the optic axis of uniaxial crystals. This effect of "linear dichroism" has already been mentioned (pp. 1505 *et seq.*). If the two coefficients differ very strongly, partially polarized light is obtained from incident natural light. A tourmaline crystal, for example, cut perpendicular to the optic axis, absorbs almost entirely the component vibrating perpendicular to the optic axis if the layer is one millimeter thick. Herapath⁶⁵ found that the biaxial crystals of iodoquinine sulfate exhibit a still stronger dichroism. A 0.1-mm. layer of herapathite absorbs almost entirely one of the two components. In order to use these crystals as polarizers, Herapath⁶⁶ tried to grow large specimens. However, another procedure proved more practical, *viz.*, the orientation of a large number of colloidal herapathite crystals. The polarizing effect is then essentially the same as that of a large single crystal. Since 1934⁶⁷ such herapathite polarizers have been produced on a large scale. The colloidal crystals, usually oriented by streaming, are kept oriented by embedding them in plastics.

More recently, herapathite seems to have been replaced by still more dichroitic crystals or molecules. Commercially, these polarizers are known primarily under the name of "Polaroid Polarizing Filters." The most recent models of Polaroid filters are claimed to produce an almost complete polarization between approximately 5200 and 6800 Å. with a loss in intensity of the less absorbed component of approximately 20%. The most important weakness of polarizers based on dichroism is that they do not and never will achieve a 100% polarization, and that they are not usable at all outside of the limited spectral region defined. Some of the optical properties of Polaroids have been investigated and reviewed and the reader maybe referred to these papers for further information.⁶⁸⁻⁷⁰

⁶⁵ W. B. Herapath, *Phil. Mag.*, **3**, 161 (1852).

⁶⁶ W. B. Herapath, *Phil. Mag.*, **6**, 346 (1853).

⁶⁷ E. H. Land, Brit. Pat. No. 412,179 (June 18, 1934).

⁶⁸ M. Grabau, *J. Optical Soc. Am.*, **27**, 420 (1937).

⁶⁹ L. R. Ingersoll, J. Winans, and E. Krause, *J. Optical Soc. Am.*, **26**, 233 (1936).

⁷⁰ J. Strong, *J. Optical Soc. Am.*, **26**, 256 (1936).

B. ANALYZER

The analyzer is used for determining the direction of vibration of linearly polarized light. Any polarizer may be used as an analyzer if the incident light is linearly polarized. Let us assume that, in figure 22 (page 1515), the incident light is linearly polarized. The prism then acts as an analyzer. No light is transmitted through the prism if the direction of vibration is perpendicular to the optic axis (direction of extinction). However, if the direction of vibration is parallel to the latter, all the light—disregarding losses by reflection—is transmitted (direction of maximum transmission). The transmission of light changes between a maximum and zero if the direction of vibration rotates through 90° . The same change is obviously observed if, instead of the vibration, the prism rotates around the direction of propagation as the axis. During a full rotation of 360° two maxima of transmission and two complete extinctions are observed. An analyzer can therefore be characterized quite generally by two orthogonal directions perpendicular to the incident light beam, the direction of maximum transmission and the direction of extinction. The angle, σ , between a vibration, V , of amplitude a , of a light beam incident upon the analyzer, and the direction of extinction OE (Fig. 28) determines the amplitude, a , of vibration, V , of the beam emerging from the analyzer:

$$a = a_i \sin \sigma \quad (12)$$

The intensity, I , of the light transmitted through the analyzer and the brightness of the analyzer field, B , are proportional to the square of the amplitude:

$$\therefore I = KI_i \sin^2 \sigma \quad (13)$$

$$B = B_s \sin^2 \sigma \quad (14)$$

where I_i is the light intensity of the beam incident upon the analyzer and B_s is the brightness of the analyzer field if $\sigma = 90^\circ$. Factor K takes into account the loss of intensity by reflection at and in the analyzer. In order to simplify, the factor K shall be considered as equal to 1.0. Equation (13) represents a modification of Malus' law:

$$I = KI_i \cos^2 \eta \quad (15)$$

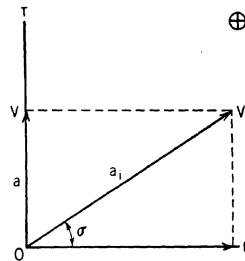


Fig. 28.—Action of analyzer upon incident linear vibration.

OT , direction of maximum transmission of analyzer. OE , direction of extinction of analyzer.

where $\eta = (90^\circ - \sigma)$. Figure 29 shows the variation of $I(B)$ with σ within the limits of 0° to 90° .

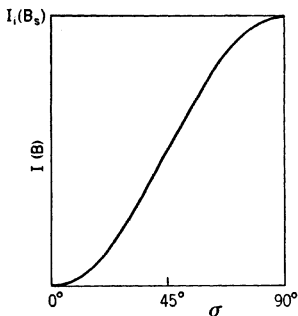


Fig. 29.—Variation of light intensity transmitted through analyzer, with angle σ between vibration of incident beam and direction of analyzer extinction.

C. POLARIZER-ANALYZER COMBINATION

The polarizer furnishes linearly polarized light from natural light. The analyzer serves to determine the change in the direction of vibration when an optically active substance is placed between polarizer and analyzer. In figure 30a, the "nicols are crossed," i. e., no light passes through the analyzer. If either prism were rotated by 90° , "parallel nicols," one would have the case of maximum transmission. Upon bringing an optically active substance between crossed nicols, light is transmitted because of the rotation of V_0 to V_1 (Fig. 1, page 1494). Extinction can be re-established by rotating the analyzer by the angle ρ_A . This angle is, for the present type of operation, identical with the optical rotation of the substance, α . Thus, if the reading on the graduated scale of the analyzer is ω_0 in the absence of the optically

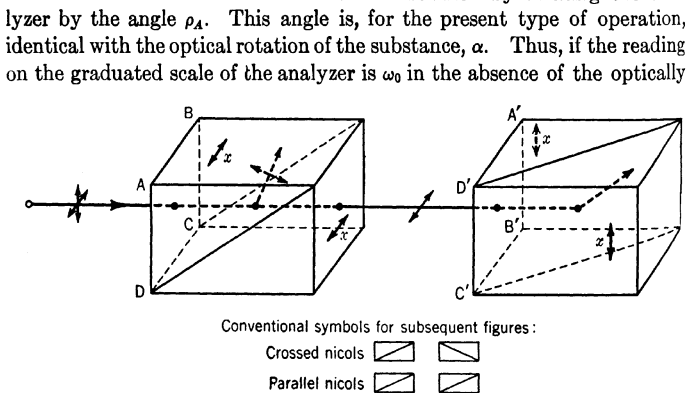


Fig. 30a.—Polarizer-analyzer combination. Case of "crossed nicols."

active substance and ω_1 in its presence (each time with the analyzer set for complete extinction):

$$\alpha = \rho_A = (\omega_1 - \omega_0) \quad (16)$$

The polarizer-analyzer combination will function satisfactorily if the

only change in V_0 is its rotation by an optically active substance. If, in addition, the state of polarization is changed, by transformation of V_0 into an elliptical vibration, complete extinction cannot be re-established (see Figs. 19, page 1512, and 41, page 1544. It is then necessary to introduce additional accessories, *viz.*, an ellipticity compensator and an ellipticity half-shade. These techniques go beyond the prescribed scope of this chapter, and they are therefore not discussed. Ellipticities, excepting those due to a Cotton effect (see the remark on page 1502), can generally be avoided or eliminated, however, in measurements of a rotatory power. One possible source of ellipticity is lenses which rarely are free of stress double refraction, except very expensive small lenses as used in condensers and objectives of polarizing microscopes built for precision measurements. It is therefore imperative to avoid the use of lenses or prisms between polarizer and analyzer. Another source of ellipticity is unsuitable or improperly handled cover glasses of polarimeter tubes (pp. 1529 *et seq.*).

For measurements of the rotatory power itself, it is unnecessary to know the direction in which a prism will transmit linearly polarized light, unless the influence of the electric or transverse magnetic field upon rotatory power is to be studied. It is very desirable, however, to know this direction if the polarizer is preceded by a monochromator (pp. 1521-1522). The direction of the diagonal plane within a polarizing prism does not necessarily give the desired information. It is, however, simple to remove the polarizer or analyzer from the polarimeter and to point it toward a window sill or a desk from which sky light is reflected. Upon rotating the prism in front of the eye, a position of minimum brightness is found. According to figure 27, in light partially polarized by reflection on a surface, the predominant component vibrates normally to the plane of incidence. Consequently, when the prism is in a position of minimum transmission, the direction of extinction is horizontal. An alternate procedure not requiring the removal of a prism from the polarimeter is as follows: The nicols are first crossed. Then the analyzer is rotated by 90° in order to obtain parallel nicols as accurately as possible. A third polarizing prism, "calibrated" as outlined, is set in front of the polarizer or between analyzer and observer and rotated until extinction is re-established. It follows from this and from an earlier discussion (page 1522) that the direction of maximum transmission of the polarizer should be perpendicular to the length of the slit of the preceding monochromator.

Defects of polarizing devices are particularly pronounced when the latter are combined into a polarizer-analyzer system. Using Fresnel's formula, we find that the maximum intensity transmitted through an analyzing Glan-Thompson prism preceded by a polarizing Glan-Thompson prism is 42.65% of the intensity of the original unpolarized beam. The only loss, beside the obvious loss of 50%, is caused by fourfold reflection. In

Polaroids, we have, in addition, the loss by absorption. According to Grabau's measurements,⁶⁸ the intensity transmitted through two parallel Polaroids (1937 type) is approximately 28% at 6600 Å. It falls to approximately 17% as one descends to 4600 Å. In the case of polarization by reflection (page 1520), a combination of two reflectors, which corresponds to parallel nicols (Fig. 27 I, page 1521), furnishes only about 1% of the original intensity. For crossed Glan-Thompson prisms, the extinction will be complete even with the sun as the light source. It will be practically complete for a set of "crossed" reflectors. Starting out with position I in figure 27, the position of "crossed reflectors" is reached by rotating mirror $A_1B_1C_1D_1$ by 90° around the incident beam, as the axis, without changing angle i (Fig. 27 II). The extinction will be fair for Polaroids unless one uses a light source of low brightness. But, even then, crossed Polaroids will transmit an appreciable amount of red and violet radiation in the newest models ("H-Filters").

D. GRADUATED CIRCLE

The graduated circle for measuring the rotation of the analyzer is divided into degrees and decimal fractions of degrees or minutes. In instruments for sugar analysis, the divisions may be in terms of sugar concentration. Regarding the latter instruments and the field of saccharimetry, the reader may be referred to the special literature.⁷¹ The graduated circle is fitted with one vernier or, for precision measurements, with two verniers. Most commonly available commercial instruments allow the reading of 0.01°, or of 0.5' and the safe estimate of 0.005°. The reading on a precision scale may be made still more accurate with a pair of parallel index lines. By turning a supplementary graduated drum, the borders of that etched line on the main scale whose exact position is to be determined are made to coincide with the lines. An instrument, manufactured by Schmidt & Haensch, which has this additional feature, is installed at the National Bureau of Standards. It allows the reading of 0.001° (3.6"). Commercial American polarimeters with this additional feature are also available.⁷² They allow a reading of 0.002°. The working principle of the index lines follows from figure 30b II, while the vernier principle is shown by figure 30b I. Instead of refining available commercial instruments in this manner, graduated circles may be used as manufactured for geodesic instruments which allow the direct reading of small multiples of seconds. Before installing such a high-precision circle in a polarimeter, one should be sure of a sufficiently high quality of the mechanical parts of the instrument. Another possibility of reading a rotation in small fractions of a minute is based on

⁷¹ F. J. Bates and associates, "Polarimetry, Saccharimetry and the Sugars," *Natl. Bur. Standards U. S. Circ. No. 440* (1942).

⁷² O. C. Rudolph, New York, N. Y.

the use of the gear principle. A toothed circle, which this time does not need to be graduated, is rotated by a slow-motion gear coupled with a counter. Such circles are not commercially available as yet, but a few of them seem to have been constructed in machine shops of various universities for use in photometers.

A precise graduation of the circle and of the vernier is a necessary but not sufficient prerequisite for precision measurements. (1) Any measurement which is to be accurate within less

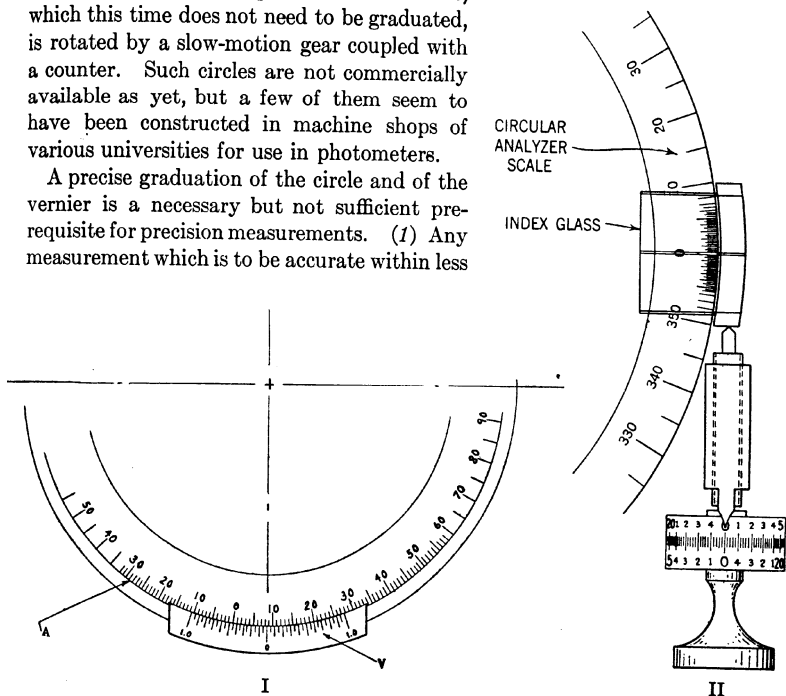


Fig. 30b.—Devices for precision readings on graduated scales (courtesy *O. C. Rudolph*). I, Vernier principle, used in most polarimeters; A, annular scale; V, vernier. II, Principle of index lines in combination with micrometerscrew, used in Rudolph polarimeter.

than two minutes must be made by means of two verniers 180° apart in order to eliminate errors due to eccentricity. (2) The instrument must have a sufficiently sensitive "null point" device (pp. 1534–1535). (3) The actual precision depends largely on the care with which the instrument is handled and operated, on the care in keeping the temperature constant, and on the sensitivity of the recording instrument, *e. g.*, the eye.

E. LIGHT SOURCES FOR MEASUREMENTS IN VISIBLE SPECTRUM

For measurements in monochromatic light, a choice is given between two possibilities: One may use a monochromatic light source in connection with a cheap monochromator of low dispersion, or with a set of filters; or

one may use a light source giving a continuous spectrum and combine it with an expensive monochromator of high dispersion. In general, monochromatic light sources emit several wave lengths strongly. However, they limit the measurements to these wave lengths, a disadvantage most pronounced for sodium lamps which in the visible have a strong emission only at 5895.9 Å. and 5889.9 Å., a doublet generally called the D line. For this reason and also because of another given above (see page 1532), it is, in principle, preferable to work with a mercury arc which emits easily visible rays at 5790.7, 5769.6, 5460.7, 4916.0, and 4358.3 Å.

A sodium lamp of the commercial type should be used with a light filter in order to exclude a faint continuous spectrum, weak sodium lines present beside the D line, and lines due to foreign gases in the lamp. With a 7% aqueous solution of potassium dichromate, a 6-cm. layer is very satisfactory, and a 1.5-cm. layer is the advisable minimum. A mercury arc, and particularly the bright high-pressure mercury lamps,⁷² must also be operated with light filters or a monochromator. The most suitable green line (5461 Å.) is isolated by a dilute solution of potassium dichromate cutting out the lines of shorter wave length and a dilute solution of neodymium nitrate cutting out the yellow lines. Any of the other four Hg lines can also be isolated by means of pairs of liquid filters.⁷³⁻⁷⁵ Optically somewhat less satisfactory, but more convenient than liquid filters, are colored glass filters.⁷⁶ Filters of dried gelatin, which are optically very satisfactory, but some of which do not withstand prolonged intense illumination, are available in a large variety.⁷⁷ Optically very satisfactory are also the "interference" filters recently perfected commercially^{77a} if used in series with a suitable Wratten filter in order to exclude undesired orders and if their maximum transmittance does not exceed 30-40%.^{77b}

With 150,000 international candles per square centimeter, the sun is the brightest light source with a continuous spectrum, discounting the Fraunhofer lines, but its use, by means of a heliostat, is limited to cloudless days. More practical are, therefore, the approximately ten times less bright carbon arcs of modern construction which prevent almost entirely wandering of the crater.⁷⁸ Next high in brightness, i. e., approximately seventy times

⁷² *General Electric Vapor Lamp Co.*, Hoboken, N. J.

⁷³ R. W. Wood, *Physical Optics*. Macmillan, New York, 1942, p. 15.

⁷⁴ F. Weigert, *Optische Methoden der Chemie*. Akad. Verlags, Leipzig, 1927, p. 62.

⁷⁵ A. Stähler, E. Thiede, and F. Richter, in *Handbuch der Arbeitsmethoden in der anorganischen Chemie*. Vol. II, part 2, de Gruyter, Berlin, 1925, p. 1530.

⁷⁶ *Corning Glass Works*, Corning, N. Y.

⁷⁷ *Wratten Light Filters*. 17th ed., Eastman Kodak Co., Rochester, 1944.

^{77a} Baird Associates, Cambridge, Mass.

^{77b} Unpublished results by K. Herrington and the author.

⁷⁸ *Bausch & Lomb Optical Co.*, Rochester, N. Y.

less bright than sunlight, are the Pointolite lamps with a tungsten arc enclosed in a glass bulb.⁷⁹ Their great advantage is a comparatively long lifetime, uniformity, and complete steadiness of the radiating area. Their quality is approached by the tungsten-ribbon filament lamps.⁸⁰ Somewhat less satisfactory, because of inhomogeneity of the radiating area, are the tungsten-coil filament lamps with a generally higher lifetime than the ribbon filament lamps.⁸⁰ In many instances, small ordinary electric light bulbs, particularly those used in automobile headlights, will be sufficient for ordinary polarimetric work. The selection of ordinary light bulbs should not be guided by the number of watts consumed, but primarily by the size of the radiating area per watt and by the working temperature of the light bulb. The higher the temperature, the brighter is the lamp per watt. An increase in temperature beyond that fixed by the manufacturer will give a higher brightness, at the cost of the lifetime of the lamp. Such an increase is brought about by raising the voltage above that prescribed for the lamp. It is not advisable, however, to exceed this voltage by more than 10%. For light sources in the ultraviolet and infrared, see pages 1578 and 1576, respectively.

F. POLARIMETER TUBES

Polarimeter tubes are covered at both ends by plane-parallel glass disks which are fused on or held in place by means of holders. For a tight fit without strain, an elastic ring, usually of rubber, is inserted between disk and holder. The tube must be placed in the light path with the reflecting surfaces of both glass disks strictly normal to the beam; otherwise the beam will be deflected and the light flux in the analyzer reduced or rendered inhomogeneous. In commercial polarimeters, the tube holders take care of the adjustment. If the glass disks are birefringent due to internal stress, they will transform linearly polarized light into elliptically polarized light (page 1512) and complete extinction between crossed nicols will be impossible. The disks are tested by crossing the nicols in the absence of the polarimeter tube and inserting the tube filled with distilled water. If the field remains completely dark, it is, nevertheless, advisable to make an additional test by rotating the tube around the light path as the axis by about 20° and 45°. If the field remains uniformly dark the disks are practically free of double refraction. Since double refraction may be caused by pressure on the glass disks, it is advisable to determine, in a separate

⁷⁹ *Philips Gloeilampen Fabrieken*, Eindhoven, Holland. *J. Biddle and Co.*, New York. *Edison Swan Electric Co., Ltd.*, London W.C.2, England. *Bausch & Lomb Optical Co.*, Rochester N. Y.

⁸⁰ *Westinghouse Electric and Manufacturing Co.*, Lamp Division, Bloomfield, N. J. *General Electric Co.*, Lamp Department, Cleveland, Ohio.

experiment, with how many turns the disk holders may be screwed in without causing noticeable double refraction. The pressure should be released at once after the detection of an effect, in order to avoid incomplete relaxation. The disks may also produce a rotation. It is therefore necessary to test, in addition, whether the angular reading found for the position of extinction is the same in the absence and in the presence of the disks. For high sensitivity, all the tests mentioned should be carried out by means of half-shade devices (see pp. 1535 *et seq.*) rather than by taking the extinction as a criterion. For windows of tubes to be used in the ultraviolet, see page 1575. The disk holders of commercial polarimeter tubes usually have a narrow opening to serve as a diaphragm in order to avoid reflection of the beam on the tube walls. These, and other diaphragms, should be painted black to avoid stray light. For precision measurements, commercially available tubes with a water jacket for thermostated water are used, because such measurements require that the liquid in the polarimeter

TABLE III
OPTICAL ROTATION OF NICOTINE IN ETHANOL^a

Nicotine, g. per 100 g. solution	Ethanol, g. per 100 g. solution	α
100.00	0	-161.42°
90.09	9.91	-141.16°
74.93	25.07	-110.62°
59.93	40.07	- 83.63°
45.08	54.92	- 59.49°
30.03	69.97	- 37.32°
14.96	85.04	- 17.46°

^a From data of H. Landolt, *Ann.*, **189**, 241 (1877). The boiling point at 74.5 mm. Hg of the nicotine used was 246.6-246.8°. d_{20}^{20} of ethanol was 0.7957. The value of α was obtained at 20° C., for the D line, with a layer of 9.992 cm.

tube be at a constant and exactly known temperature. A special water-jacketed tube developed by the National Bureau of Standards^{81a} makes it possible to study the rotatory power at any temperature between 0° and 100° C. without the risk of leakage or of a distortion of the optical field through temperature gradients. A special cap, to prevent fogging of the windows at low temperature, has also been developed.^{81a} Instead of using water-jacketed polarimeter tubes, the polarimeter itself may be placed in a thermoconstant room. The construction of such a room has been described in detail by Bates.^{81b} For measurements of optical activity of substances available in amounts too small for a standard-size polarimeter tube, a capillary tube has been devised which requires only 1.8-4.6 mg. of substance in 0.64-1.35 g. of solution. The error in α is claimed to be as small

⁸¹ F. J. Bates and associates, *loc. cit.* (footnote 71). (a) pp. 103-105. (b) pp. 97-102.

as $\pm 2\%$.⁸² A device by means of which a polarimeter tube, made of hard rubber, can be agitated in a horizontal and circular motion has been described by Straub⁸³ for use with protein solutions.

In order to determine the optical length of a polarimeter tube, one may measure the rotation of a liquid or a solution of known strong rotatory power at a constant and accurately known temperature. The rotatory power of nicotine in ethyl alcohol is so strong that the thickness of a layer can be determined with an error $< \pm 0.01$ mm. Data are given in table III. The variation of α with the concentration is practically linear. The optical length of a polarimeter tube varies slightly with temperature. If the optical length, i. e., the layer thickness, b , is determined at 20° C., it may be calculated for any temperature, T , from the equation:

$$b_T = b_{20}[1 + \mu(T - 20)]$$

where μ is the linear expansion coefficient of the material. The value of μ for a glass tube is 8×10^{-6} .

3. Visual Polarimetry without Compensator in Monochromatic Light

A. SOME ELEMENTS OF PHYSIOLOGICAL OPTICS

Since the eye is the recording instrument in visual polarimetry, it is necessary, to use it to best advantage, to be familiar with some of its relevant characteristics.

Relative Visibility.—The sensitivity of the eye for a given wave length can be measured in terms of visibility at this wave length, which is defined by the ratio of luminosity to radiant power of the light source, i. e., by lumen/(erg sec.⁻¹). The extreme limits of visibility reached experimentally, under optimum conditions, are 8350 and 3650 Å., respectively. The visibility at the extremes of the given range is very small compared with that in the middle. It is a million times smaller at 3650 Å. than at 5600 Å. For brightnesses corresponding to that of diffuse daylight, the maximum visibility—photopic vision—is at 5550 Å., as shown in curve *I* of figure 31 which gives averages compiled by Gibson and Tyndall.⁸⁴ The data are given in terms of relative visibility, i. e., the maximum visibility at 5550 Å. is set equal to 1.0.

At low brightness levels when the eye is said to possess scotopic, or dark, vision, the wave length of maximum sensitivity is shifted toward the blue (Purkinje phenomenon).^{*} The transition from daylight to scotopic vision begins at about a brightness of 0.1 foot-lambert (see p. 1408), which corresponds to the brightness of a white surface in a room receiving barely enough daylight for reading or writing without strain to the eyes. Figure 31 (curve *II*) shows the spectral distribution of sensitivity of the eye when the brightness of the field observed is, at best, 56 micro

⁸² N. von Bekesy, *Biochem. Z.*, **312**, 103 (1942).

⁸³ J. Straub, *Chem. Weekblad*, **31**, 465 (1934).

⁸⁴ K. S. Gibson and E. P. T. Tyndall, *Natl. Bur. Standards U. S. Sci. Paper*, No. 475, 19, 131 (1923-1924).

foot-lamberts,^{85-86a} which corresponds to the brightness of the clear night sky in absence of the moon. The maximum sensitivity is this time reached at 5110-5113 Å., the latter value being generally accepted as the standard for scotopic vision.

In polarimetry, one will frequently be confronted with brightnesses below the critical value for which curve *I* (Fig. 31) holds. If, therefore, measurements are to be made at a single wave length selected by the observer, a wave length within the range of highest sensitivity should be chosen. Even at a high brightness, the

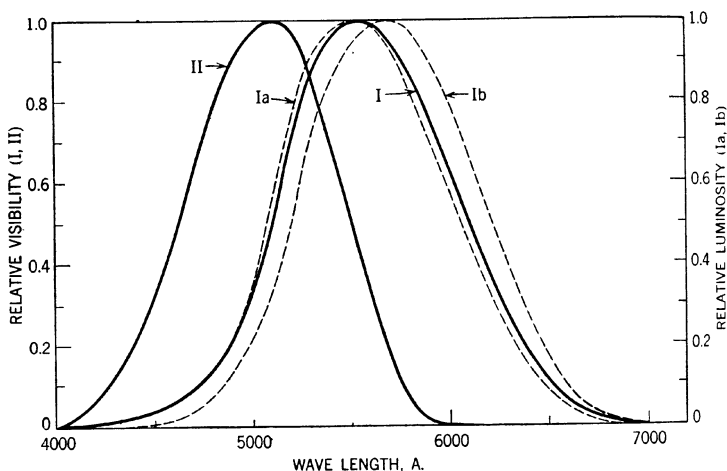


Fig. 31.—Relative visibility and luminosity.

Curve *I*, relative visibility for daylight vision (data of Gibson and Tyndall); *Ia* relative luminosity of sun in zenith recorded at sea level. *Ib*, relative luminosity of commercial tungsten-filament lamp; *II*, relative visibility for scotopic vision (data of Luckiesh and Taylor).

eye is definitely more sensitive to the green line of the mercury arc than to the yellow sodium lines of equal absolute brightness; and at low brightnesses the differences are much greater. A mercury arc is therefore preferable to a sodium lamp if the radiant power of the former at 5461 Å. is equal or slightly inferior to the radiant power of the latter at the D line. Particularly for sources with continuous spectrum, it should be kept in mind, however, that the relative visibility, though it is the decisive factor, is not the only factor to be considered in the choice of the most advantageous wave length. If the radiant power of the light source varies strongly

⁸⁵ M. Luckiesh and A. H. Taylor, *Illum. Eng.*, **38**, 189 (1943). A. H. Taylor, *Illum. Eng.*, **38**, 89 (1943).

⁸⁶ K. S. Weaver, *J. Optical Soc. Am.*, **27**, 36 (1937). S. Hecht and R. E. Williams, *J. Gen. Physiol.*, **5**, 1 (1922).

^{86a} N. I. Pinegin, *Nature*, **155**, 20 (1945).

with wave length, the actual impression of brightness in photopic vision, *i. e.*, the luminosity, may reach its peak at a wave length somewhat lower or higher than 5550 Å. Curves *Ia* and *Ib* give the variation in luminosity with wave length for the sun—observation at sea level with the sun in the zenith—and for a commercial gas-filled tungsten-filament lamp, respectively. Detailed calculations of luminosities of a radiator within the range of color temperatures corresponding to that of tungsten lamps employed in experimental work (2000° to 3120° K.) have been carried out by Skogland.⁸⁷

Adaptation of the Eye.—At a given wave length, the eye is more sensitive toward a newly introduced brightness, B_{II} , the lower the brightness, B_I , to which it was previously adapted. A certain time elapses before the eye ceases to be dazzled if $B_{II} \gg B_I$, and before B_{II} is perceived if $B_{II} \ll B_I$. In the latter case, full sensitivity to B_{II} requires quite some time. The case $B_{II} \ll B_I$ is the common situation in polarimetry. The eye is first adapted from daylight to the lower brightness of the polarimeter field and later on re-adapted to it after an action at higher brightness, as during the reading of a scale or the recording of results on paper. Table IV gives data of Blanchard⁸⁸ showing the time, *t*, required before the eye, previously adapted to a brightness B_I , is just able to perceive a much lower brightness, B_{II} .

TABLE IV
RATE OF ADAPTATION OF THE HUMAN EYE IN VARIOUS SPECTRAL REGIONS^a

<i>B_I</i> :	White				Blue	Green	Yellow	Red
	0.1	1.0	10	100	0.1	0.1	0.1	0.1
<i>t</i>	-log B_{II}							
0 sec.	2.79	2.20	1.60	0.90	2.82	2.69	2.61	2.32
2 sec.	4.13	3.27	2.53	2.00	4.36	4.39	4.17	2.98
5 sec.	4.50	3.79	3.08	2.46	4.91	4.82	4.41	3.37
10 sec.	4.75	4.15	3.54	2.64	5.27	5.11	4.65	3.57
1 min.	5.32	5.06	4.61	3.84	5.81	5.56	5.09	3.80
2 min.	5.52	5.22	4.83	4.12	6.00	5.70	5.24	3.92
5 min.	5.68	5.52	5.22	4.76	6.23	5.80	5.39	4.02
10 min.	5.70	5.68	5.59	5.38
60 min.	6.06	6.04	6.01	5.97

^a Data of Blanchard. Symbols in this table are explained in the text. Both B_I and B_{II} are brightnesses, expressed in millilamberts (1 millilambert = 0.000354 candle per sq. cm.).

In the light of these facts, the polarimeter should be operated in a darkened room, with a shielded light source, and measurements should not be started before 10 minutes after darkening. The necessary periodic illumination of scales and notebook should be as low as possible without strain for the eyes. Small light bulbs, operated at a voltage lower than that indicated at the mounting and shielded with red paper, should be installed near the area to be illuminated in order to allow only

⁸⁷ J. F. Skogland, *Natl. Bur. Standards U. S. Misc. Pub. No. 86* (1929).

⁸⁸ J. Blanchard, *Phys. Rev.*, **11**, 81 (1918).

indirect light to reach the eye. The eye will then become almost completely re-adapted to dark within 10 seconds. If a weakly absorbing substance exhibits strong rotatory power, the high intensity transmitted through crossed nicols may blind the eye. It is then advisable to rotate the analyzer to near-extinction and to wait with the final measurement until the eye has recovered its sensitivity.

The sensitivity of a well-adapted eye is very often underrated. According to Hecht,⁸⁹ the energy necessary for the production of the minimum visual effect, by means of an eye well adapted to the dark, is 5-8 light quanta. To that effect, it is necessary that 54-148 quanta are incident upon the cornea of the eye, the balance being lost before the light reaches the visual purple of the retina. In order to produce a detectable grain of silver on a photographic plate, 50-100 quanta are necessary.⁹⁰ In order to liberate one photoelectron from a photosensitive layer, 15-50 quanta are necessary; and 100,000 quanta per second are required in order to produce a steady photoelectric current (page 1588). The sensitivity of the dark-adapted eye is therefore not surpassed by any artificial recording device.

Ability of the Eye to Distinguish between Brightnesses.—The simplest method of measuring a rotation would be by rotating the analyzer until complete extinction is established. If the sensitivity of the eye were the only important physiological factor, it would be possible to achieve a remarkably high accuracy in such measurements. A minute deviation of the analyzer from the position of extinction would be detectable. However, success in finding the position of complete extinction would require that the eye also *remember quantitatively* the various degrees of brightness perceived during a series of trials with analyzer positions slightly off the position of the true minimum. Unfortunately, small differences in *gradually varied sensation* which are involved in the present instance are not remembered well, whereas *fluctuations in sensation* near the absolute limit of visibility of the human eye are registered with such a remarkably high sensitivity that sudden differences, of the order of magnitude of 10 quanta, can be detected and estimated by particularly well-trained persons. The error in finding the position of complete extinction is generally defined by an uncertainty of not less than 3 minutes on the graduated scale. In cheap instruments, the graduated circle may not allow a better reading. It is then superfluous to use a principle other than that of extinction. For precision measurements, however, the principles of the half-shade or of the fringes are used which do not require the recollection of brightnesses.

In the methods based on the half-shade principle, the determination of minimum brightness is replaced by a determination of equal brightness in adjacent fields. According to the psychophysical law of Weber-Fechner,

⁸⁹ S. Hecht, *Am. Scientist*, **32**, 159 (1944), the extensive literature references given there, and *J. Optical Soc. Am.*, **32**, 42 (1942).

⁹⁰ K. O. Kiepenheuer, *Z. Physik*, **107**, 145 (1937).

the eye is able to distinguish between two brightnesses, B_I and B_{II} , if their logarithmic difference is not smaller than 1-2%, that is, if:

$$\log (B_I/B_{II}) \sim \log 1.01 \sim 0.004 \quad (17)$$

This law holds for moderate brightnesses but not for brightnesses that dazzle the eye adapted to diffuse daylight, or for brightnesses equivalent to, or lower than, the brightness of a white surface in moonlight. The decline in the differentiating power of the eye with declining brightness is more pronounced at longer wave lengths. If, at a given low brightness, the error in differentiation is 30% at 5050 Å., it rises to 80% at 6050 Å.

In some of the methods using the fringe principle, the brightness or darkness of a stationary fringe is varied until it equals that of the field, *i. e.*, the fringe disappears. From the point of view of physiological optics, the problems are the same as in the half-shade method. In other methods, one observes the linear displacement of a fringe or of fringes in a contrasting field. In this case, additional phenomena of physiological optics are involved. Only a few practical aspects can be discussed here. The fringes encountered in polarimetry are generally without sharp boundaries. In order to measure a displacement accurately, it is necessary that the boundaries *appear* as sharp as possible. If the fringes are broad, it is, in addition, desirable that they *appear* narrow, because the position of the center of a fringe is often the criterion in fringe measurements. As discussed extensively by Helmholtz,⁹¹ the impressions of both sharpness and narrowness are furthered by adapting the eye well, by focusing as sharply upon the fringe as possible, and by making the objective dark-white contrast—or color contrast—as strong as possible (without blinding the eye) through use of as powerful as possible a light source.

An interesting, but fragmentary, discussion of the precision of the half-shade and fringe methods has been made by Yves Le Grand.⁹²

B. HALF-SHADE METHOD

The half-shade is a device which transforms complete extinction, in the case of crossed nicols, into equal illumination of two adjacent fields. It will be seen that both fields then generally appear very dim. This effect has led to the coining of the term "half-shade," or "penumbra," either corresponding to the French *pénombre*. Since no shadow of anything is involved, the often preferred, but incorrect, term "half-shadow" shall be disregarded. The term "isophotostatic" device⁹³ is, theoretically, preferable to "half-shade," but is too cumbersome to compete with the latter.

⁹¹ H. Helmholtz, *Handbuch der physiologischen Optik*. Voss, Leipzig, 1867, especially pp. 135, 323.

⁹² Y. Le Grand, *Rev. optique*, 12, 145 (1933).

⁹³ N. Deer, *Intern. Sugar J.*, 37, 421 (1935).

The half-shade principle is explained by figure 32, which illustrates the action of two adjacent Glan-Thompson prisms, *A* and *B*. Dotted lines across the prisms represent the directions of extinction for linearly polarized light. The inner circle is the circumference of a rotatable mounting for the two prisms. The pointer protruding from the rotatable mounting serves to indicate on the outer, fixed, circle the positions of the mounting when the fields of prisms *A* or *B* are brightest (*b*) or completely dark (*d*), respectively. The symbols at the peripheral part of the outer circle refer to *A*, those at the inside, to *B*. In figure 32*a*, the dotted directions of ex-

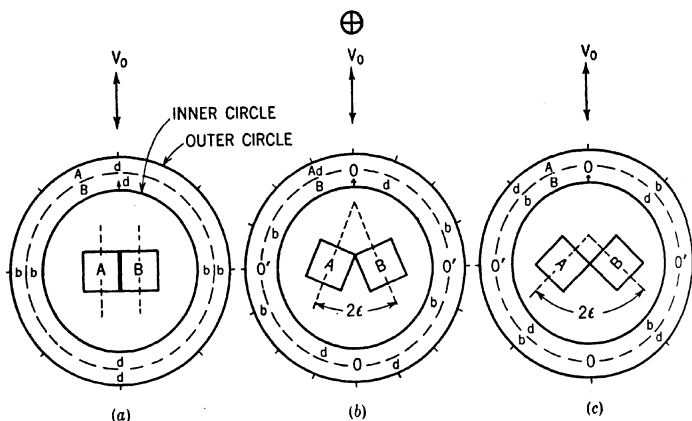


Fig. 32.—Half-shade principle.

inction are parallel and the double prism functions as a single analyzer. In figure 32*b*, where the directions of extinction form a "half-shade angle," 2ϵ , of 45° , a rotation of -22.5° will lead to complete extinction in *A*, and a rotation of $+22.5^\circ$ to complete extinction in *B*. The half-shade angle thus is the smaller of the two possible angles, 45° and $(180^\circ - 45^\circ)$, which characterize the angular difference for complete extinction in *A* and *B*. In the intermediate "zero" position, 0, both fields are equally dim.

The change in brightness in *A* and *B* with a rotation, ρ_A , of the rotatable analyzer mounting is illustrated by figure 33. It will be noticed that at right angles to 0 the brightness of the two fields is also equal, $0'$, but far higher than at 0, and that $0'$ is less suitable as a practical zero position (see Fig. 33). Depending on the angle, 2ϵ , the brightness of the double field in the zero position, 0, will vary between zero ($2\epsilon = 0$, Fig. 32*a*) and half the value at maximum transmission ($2\epsilon = 90^\circ$, Fig. 32*c*). In the latter

case, the brightness in position 0 is equal to the brightness in position 0' (Fig. 32), *i. e.*, in monochromatic light, the position equivalent to crossed nicols is identical with that of parallel nicols.

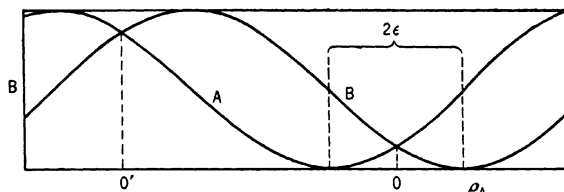


Fig. 33.—Variation in brightness of the two analyzer fields with rotation, ρ_A , of analyzer mounting of figure 32. Half-shade angle of 45° .

Figure 34 shows that a small half-shade angle should be chosen for maximum precision. $B_{Cr.}$ is the critical brightness below which the eye no longer detects a brightness difference, ΔB , of 1–2%. $B_{Eq.}$ is the brightness at the intersection of the two curves—brightness equality in the two fields. For the sake of demonstration, ΔB is assumed to be $0.2 B_{Eq.}$. The range of rotation, $2\Delta\rho$, within which the eye cannot distinguish between the

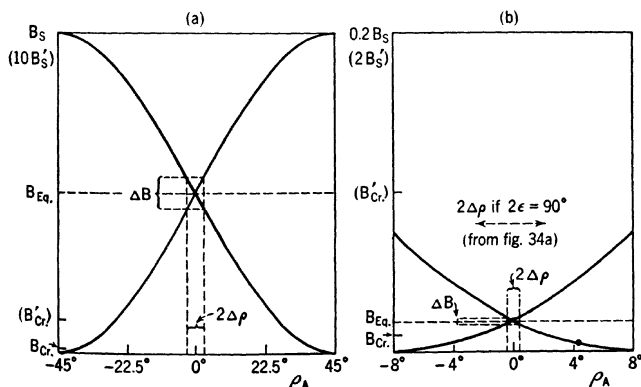


Fig. 34.—Small half-shade angle vs. large half-shade angle: a, half-shade angle of 90° ; b, half-shade angle of 16° .

brightnesses of the two fields is much larger for a large half-shade angle (Fig. 34a) than for a small one (Fig. 34b). The smallest half-shade angle is set by the requirement that the brightness in the zero position, $B_{Eq.}$,

not be lower than B_{Cr} . If the maximum brightness of the analyzer field, B_s , is smaller, B'_s (less powerful light source), then the critical brightness may have the value B'_{Cr} . It is obvious from figure 34 that the large half-shade angle (Fig. 34a) will give more accurate results than the small angle (Fig. 34b). Hence, the half-shade angle should be variable if the brightness of the analyzer field is variable, *i. e.*, if the transparency of the substance investigated or the layer thickness varies, if the wave length is to be varied, or if the brightness of the light source changes.

The precision of the half-shade method follows from table V, which gives data calculated by Landolt⁹⁴ on the assumption that ΔB , perceptible to the eye, is $0.02 B_{Eq}$ for all the half-shade angles. This assumption is cer-

TABLE V
LANDOLT DATA FOR HALF-SHADE ANGLE, 2ϵ , AND UNCERTAINTY, $\pm \Delta\rho$,
IN DETERMINATION OF OPTICAL ROTATION*

2ϵ	$\pm \Delta\rho$
0.5°	$4''$
1°	$9''$
2°	$18''$
4°	$36''$
6°	$54''$
8°	$72''$
10°	$1.5'$

* Since the commercial polarimeters commonly available do not have circular scales allowing a reading better than 0.01° , the use of half-shade angles smaller than 4° is only indicated for polarimeters provided with high precision scales, or special precision verniers.

tainly not fulfilled for the two smallest half-shade angles, excepting possibly the case in which the sun is the light source. The data are expressed in terms of $\Delta\rho$ which is the physiological uncertainty involved in the determination of the rotation, ρ :⁹⁴⁻⁹⁶

$$\pm \Delta\rho = \frac{\epsilon}{4} \frac{\Delta B}{B_{Eq}} \quad (18)$$

It follows from this equation that the uncertainty, $\Delta\rho$, is proportional to the half-shade angle as long as the Weber-Fechner law holds, *i. e.*, as long as $\Delta B/B_{Eq}$ is a constant, and that it increases, for a given half-shade angle, if the Weber-Fechner constant increases (see pages 1534-1535).

The simplest practical analyzer with half-shade effect is the Cornu

⁹⁴ H. Landolt, *Das optische Drehungsvermögen organischer Substanzen*. Vieweg, Braunschweig, 1898, pp. 304, 305.

⁹⁵ A. Wüllner, *Lehrbuch der Experimentalphysik*. Vol. IV, *Die Lehre von der Strahlung*. Teubner, Leipzig, 1899, pp. 966-967.

⁹⁶ See particularly L. Chaumont, *Ann. phys.*, **4**, 61 (1915); **5**, 17 (1916).

modification of the Jellett prism.^{97, 98} It is made by sawing a Glan-Thompson prism in half, along AB in figure 35, grinding one of the halves down to AC and cementing the two parts. Since the half-shade angle is invariable,

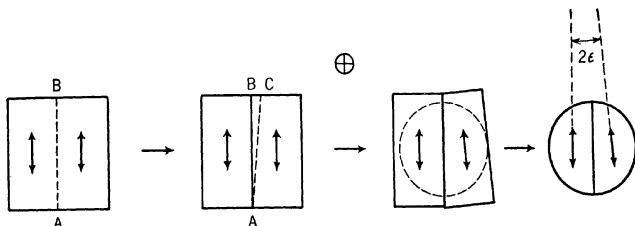


Fig. 35.—Jellett-Cornu prism. Arrows indicate direction of extinction.

the Jellett-Cornu prism is satisfactory only if the light flux arriving in front of the prism is approximately the same under all experimental conditions and, in addition, of the order of magnitude for which the fixed half-shade

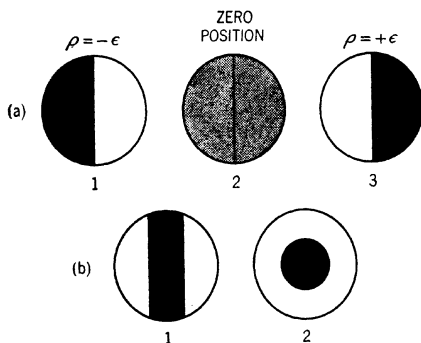


Fig. 36.—Aspect of half-shade field: (a) changes in aspect during the search for field equality; (b) aspect of special half-shade fields, off position of field equality.

angle is most suitable. Jellett-Cornu prisms are recognized by the fine dividing line across their frontal face. Figure 36a gives the aspect of the optical field, which is usually made circular by a diaphragm, for the zero

⁹⁷ J. H. Jellett, *Researches in Chemical Optics*, Dublin Univ. Press, 1875. See also *Brit. Assoc. Advancement Sci. Rept.*, 29, 13 (1860).

⁹⁸ A. Cornu, *Bull. soc. chim.*, 14, 140 (1870).

position (2), after rotation by $-\epsilon$ (1), and after rotation by $+\epsilon$ (3). The virtual image of one analyzer face is usually observed by means of a telescope.

Double image prisms (see pages 1519 *et seq.*) represent another type of simple analyzer with half-shade effect. The half-shade angle is 90° since the two emerging components are polarized at right angles. These prisms play an important role in nonvisual polarimetry, where large half-shade angles are desirable, particularly in photoelectric polarimetry (page 1592), but are without major interest as half-shade analyzers in visual polarimetry, where small half-shade angles are required.

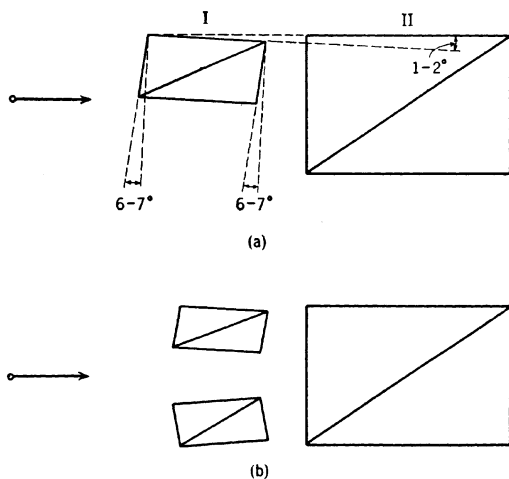


Fig. 37.—Lippich prism: (a) double field arrangement; (b) triple field arrangement.

In most cases, the half-shade effect is brought about by setting an appropriate device in front of a simple analyzing prism. The telescope is focused upon the device, not, as above, upon the analyzer. With the exception of a few cases mentioned on pages 1542 and 1545, the field is the same as in figure 36a.

The best "compound analyzer," *i. e.*, analyzer with additional half-shade device, is the Lippich prism.⁹⁹ Half of the field of a simple analyzer, II (Fig. 37a), is covered by a preceding analyzer, I, of smaller size. The

⁹⁹ F. Lippich, *Sitzber. Akad. Wiss. Wien, Math.-naturw. Klasse, Abt. IIa*, 91. 1059 (1885).

directions of extinction of the two prisms form a small angle (see Fig. 38). Figure 37b shows a commercially favored type of Lippich prism in which the field is divided into three parts by setting two small prisms in front of the main analyzer; the aspect of the field is, in this case, as shown in figure 36b, view 1, assuming a rotation by ϵ . The small prism of the two-field Lippich prism is arranged in a slightly slanting position, $1-2^\circ$, in order to obtain a well-illuminated and sharp dividing line of the two fields. For the same reason, the faces of the small prism are corrected by $6-7^\circ$. The half-shade angle can be varied by rotating—within a limited range—the small prism and the main prism independently one from the other. For this purpose, polarimeters fitted with a Lippich prism usually have a lever on the mounting (see Fig. 53, page 1553).

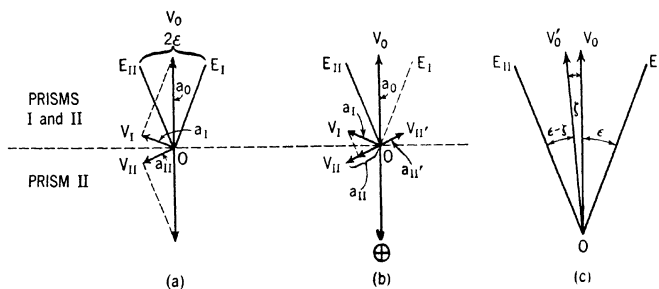


Fig. 38.—Principle of Lippich half-shade method (double-field arrangement).

In figure 38, it is assumed that the directions of extinction, OE_I and OE_{II} , of prisms I and II, respectively, form a half-shade angle, 2ϵ , of 45° , and that OE_I and OE_{II} are symmetrical with respect to V_0 . If the Lippich prism could function like the Jellett-Cornu prism, field equality would be expected in this position; but such is not the case. To demonstrate this fact and the important reason for it, we propose to find the directions and amplitudes of the vibration, V_{II} (Fig. 38a), transmitted through the lower half of prism II, which is not preceded by prism I, and of the vibration, V_I , which is transmitted through prism I, disregarding at first the subsequent transmission through prism II. The amplitudes are obtained according to the procedure used for figure 28. It is apparent that the amplitudes, a_I and a_{II} , are equal. V_I is now altered during the subsequent passage of the beam through prism II. The resulting vibration, V_{II}' (Fig. 38b), is parallel to V_{II} but its amplitude, a_{II}' , is smaller than a_{II} . The two half-fields of the Lippich analyzer are therefore not equal in brightness. In order to make them equal, a_I must be increased by increasing the angle between V_0 and OE_I to V_0OE_I by an increment, ζ (Fig. 38c).

The situation and the working principle of the Lippich prism are summarized in figure 38c. If the incident light vibrates parallel to either OE_I or OE_{II} , the respective half-field is completely dark. The vibration, V'_0 , which leads to field equality, deviates by the angle ζ from the position of symmetry with respect to OE_I and OE_{II} , that is, it deviates by the angle ζ from vibration V_0 , which would be extinct if a simple analyzer were used. The half-shade effect of the Lippich prism may therefore be called "asymmetrical," while the half-shade effect of the Jellett-Cornu prism may be called "symmetrical." The asymmetry as such is of no importance in the measuring of rotations since ζ obviously does not vary with α . However, one should remember that ζ varies with the half-shade angle. A zero position

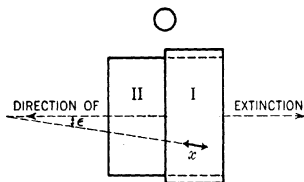


Fig. 39.—Laurent arrangement.

determined for a given half-shade angle is therefore not strictly valid for another half-shade angle.

The Lippich prism has another peculiarity which, if overlooked, may cause errors. The light traversing both II and I is weakened more by reflection than that traversing I only. The difference changes the angle ζ by $\Delta\zeta$ to ζ' (not shown in Fig. 38), and $\Delta\zeta$ is

likely to be even larger than ζ itself if the half-shade angle is small. As a result, the zero position will vary with the wave length because of the spectral variation in the reflecting power of the prisms. A zero reading taken in absence of the optically active substance holds, therefore, strictly only for the wave length and the half-shade angle used.

Another, often used, compound analyzer with variable half-shade angle has been introduced by Laurent.^{100, 101} It is sturdier and somewhat cheaper than the Lippich prism. The Laurent compound analyzer, shown schematically in figure 39, consists of a Glan-Thompson prism, II, half of which is preceded by a plane-parallel plate, I, of quartz cut parallel to its optic axis. In some cases, a circular strip of quartz is cemented onto a glass plate.¹⁰² The aspect of the field is then that of figure 36b, view 2. This construction has some advantages.

Quartz is optically active toward light propagated parallel to its optic axis (see page 1545), but birefringent toward light propagated perpendicular to its optic axis. The half-shade effect of the Laurent compound analyzer is therefore based on the principle of double refraction. With the incident beam linearly polarized and the angle of incidence 0° , the light emerging

¹⁰⁰ H. Laurent, *J. phys.*, **3**, 183 (1874).

¹⁰¹ U. Gayon, *J. phys.*, **8**, 164 (1879).

¹⁰² H. Heele, according to E. Gumlich, *Z. Instrumentenk.*, **16**, 269 (1896).

from the quartz plate is linearly polarized only if the phase difference equals π (180°) or a multiple of π (see Fig. 20, page 1513). For even multiples of π , the direction of the vibration emerging from the plate is identical with that of the original vibration. For a phase difference of π or an uneven multiple of π , the direction of vibration, V_I , of the beam emerging from the quartz plate differs from the original direction of vibration, V_0 . V_I and V_0 are symmetrical with respect to the optic axis. The angular displacement, 2ϵ , increases with angle γ between V_0 and the optic axis up to an angle of 45° . The latter angle leads to the maximum angular

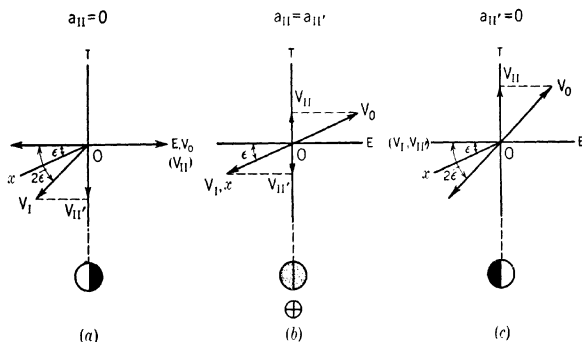


Fig. 40.—Principle of Laurent half-shade method.

Vertical coordinate represents dividing line between uncovered half-field (right) and quartz-covered half-field of Glan-Thompson prism. V_I , direction of vibration after light has traversed quartz plate and before it traverses prism; V_{II} , direction of vibration of light traversing prism only; $V_{II'}$, alteration of V_I by subsequent passage through prism.

displacement of 90° (Fig. 20). The quartz plate in the Laurent compound analyzer causes such a symmetrical displacement of the incident linear vibration. From the optical constants of quartz (n_e , 1.553; n_o , 1.544) for the D line, it follows that a thickness of 32 microns or of an uneven multiple of 32 microns is required in order to obtain a phase difference of π , or of an uneven multiple of π , respectively, in work with the D line.

Figure 40 demonstrates the result of the symmetrical displacement. The part of the Glan-Thompson prism covered by the plate is at the left; the other part of the prism is at the right of the vertical coordinate. In figure 40a, V_0 is parallel to the direction of extinction of the prism, OE ; in figure 40b it is parallel to the optic axis of the quartz plate; and in 40c it is twice as far from OE as from Ox in terms of angular distance. Case b represents the position of field equality. The half-shade angle, 2ϵ , 45° in

the example, is equal to twice the angle between OE and Ox . The half-shade effect is symmetrical, except for the disregarded asymmetry introduced by unequal reflections in the two half-fields, and can be varied by rotating the quartz plate while the prism is kept in position.

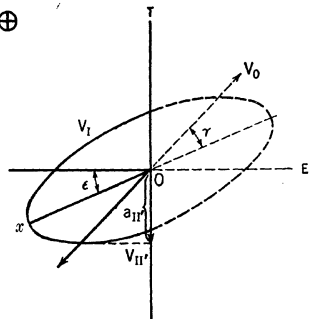


Fig. 41.—Deficiency of Laurent arrangement if used with light of unsuitable wave length. Changes for light beam which traverses prism only are omitted.

tion into a linear vibration, $V_{II'}$, of amplitude $a_{II'}$. For a given angle, γ , a linear vibration will be transmitted whatever the orientation of the ellipse with respect to the direction of extinction, OE . It will be impossible, therefore, to obtain complete extinction in that part of the analyzer field which is preceded by the quartz plate. A change of γ , on the other hand, changes the axial ratio, c/b , of the ellipse (Fig. 20). The ratio increases to infinity as γ decreases to zero. In the latter limiting case, the linear vibration passes the quartz plate unaltered. The

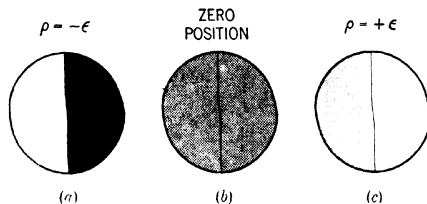


Fig. 42.—Variation in aspect of half-shade field if Laurent arrangement is used with light of unsuitable wave length.

phenomena just described lead to a particular kind of asymmetry of the half-shade effect. The zero position (Fig. 40b), characterized by field equality, remains unaffected by the state of vibration of V_I . In positions a and c (Fig. 40), however, the

light transmission through the part of the prism preceded by the quartz plate (left-hand part of the drawings) will always be finite if V_1 is an elliptical or circular vibration. A more detailed picture may be obtained by visualizing a figure 40c in which the left-hand part is replaced by the left-hand part of figure 41. Figure 42 gives the actual aspects of the field for positions equivalent to those in figure 36a (page 1539). The accurate determination of the zero position is somewhat difficult because of the absence of pronounced contrast if the analyzer rotates from the position of field equality (position b) toward position c. The uncertainty in determining the position of field equality is therefore larger than at the wave length, λ^* , for which the quartz plate is a "half-wave plate." Assuming that λ differs from λ^* by 500 Å., the error will be 50% larger than defined by equation (18). If the phase difference at λ^* is 3π , as is usual in commercial Laurent compound prisms using quartz, even a difference of $(\lambda - \lambda^*) = \pm 150$ Å. will lead to a 50% increase in error. It is therefore preferable to use a quarter-wave mica plate instead of a commercial Laurent quartz plate.

The symmetrical displacement of the direction of vibration by a half-wave plate is equivalent to a rotation. Consequently, a half-shade effect can also be produced by superimposing upon half of the analyzer field an optically active substance, *e. g.*, a plate of quartz cut perpendicular to the optic axis. A number of such half-shade arrangements are on the market, *e. g.*, those designed by Macé de Lepinay and Wright. Their use is not advisable for precision measurements.

Much more preferable is the half-shade arrangement by Nakamura,¹⁰³ a modification of the Soleil biquartz (pp. 1560 *et seq.*). A thin plate of left quartz, Q_l , and an equally thin plate of right quartz, Q_d , are mounted, side by side, in front of the analyzer. Nakamura recommends a thickness of $1/5$ to $1/25$ mm. (rotation for D line and 20° C., 4.4° and 0.9°, respectively). The half-shade angle is therefore equal to twice the rotation of either plate. A half-shade angle of 2°, 4°, and 6° requires a biquartz 46, 92, and 138 microns thick, respectively (D line). Figure 43 shows the direction of vibration before and after the light beam has traversed the twin plate. This compound analyzer has an advantage over the Lippich and the Laurent compound analyzers in that

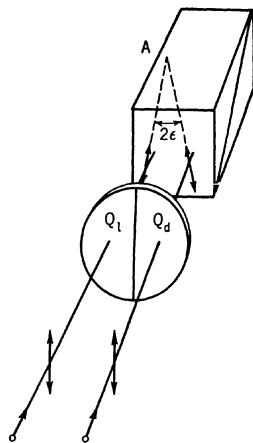


Fig. 43.—Nakamura half-shade.

Q_l , levorotatory quartz plate
 Q_d , dextrorotatory quartz plate

¹⁰³ S. Nakamura, *Centr. Mineral. Geol.*, 1905, 267.

the thickness of the total quartz layer and the angle, β , must be known, or the apparatus must be calibrated by means of substances of known rotation. The sensitivity of the Senarmont arrangement equals that of half-shade devices.

Savart Polariscope.—If the quartz plate of a Laurent compound prism (page 1542) extends over the whole field of the analyzer, the incident linear vibration of monochromatic parallel light forms an angle of 45° with the optic axis, and the optic axis of the plate forms an angle of

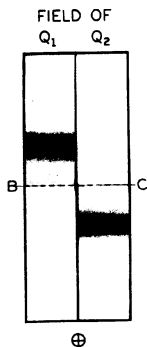


Fig. 46.—Field aspect with Senarmont fringe arrangement.

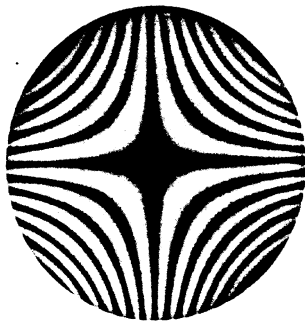


Fig. 47.—Fringes in convergent monochromatic light produced by a plane-parallel birefringent crystal plate between crossed nicols if optic axis is at 45° to direction of analyzer extinction and perpendicular to incident beam.

45° with the direction of extinction of the analyzer, then the phase difference of 180° , produced by the plate, will cause the field to appear bright between crossed nicols, and dark between parallel nicols. If the quartz plate is twice as thick, the phase difference, now 360° , will lead to extinction between crossed nicols and to maximum transmission between parallel ones.

In convergent light, under otherwise equal conditions, the phase difference will vary across the plate. Consequently, the plate will not have a uniform aspect between two nicols if the eye is focused upon it, but the field will be intersected by hyperbolic fringes (Fig. 47). This means that the points of equal phase difference lie on hyperbolic curves. The asymptote forms an angle of nearly 45° with the optic axis of the plate. The transition from crossed to parallel nicols leads to a transition of the

dark into bright hyperbolae, and to a transition of the bright parts of the field into dark ones. The contrast is most pronounced for an angle of 45° between optic axis and V_0 . It becomes weaker for smaller and larger angles; and the hyperbolae disappear completely if the angle is 0° or 90° . Unless the plate is very thin, the fringes are too numerous, too close to each other, and too narrow to be distinct without magnification. This difficulty can be overcome by using two thick plates of equal thickness. Superimposed with their optic axes at right angles (Fig. 48a), they exhibit fringes identical with those of a single thin plate.

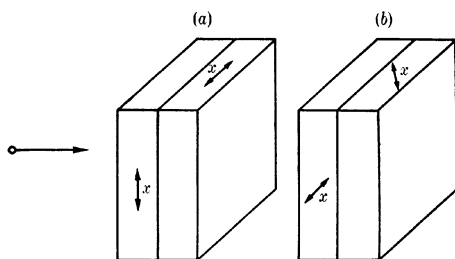


Fig. 48.—Fringe arrangement with pair of quartz or calcite plates, using effect of double refraction.

a, fringe arrangement giving hyperbolic fringes; *b*, Savart plate giving straight fringes. For aspect of fringes in *a* and *b*, see figures 47 and 49, respectively.

In the double plate introduced by Savart¹⁰⁶ the inconvenience of having curved fringes is eliminated. Each of the two plates is cut under an angle of 45° to the optic axis, so that in the crossed plates the optic axes form an angle of 90° as above (Fig. 48b). The position of the optic axes with respect to the direction of vibration of the incident light, however, differs from that in the preceding double plate. As a result, instead of hyperbolic fringes, straight fringes are formed which are parallel to each other and to the direction of transmission of the analyzer (Fig. 49a). The fringes are dark between crossed nicols and bright between parallel nicols, and are most pronounced if the planes of the principal sections of the plates form an angle of 45° with V_0 . If this angle decreases, the fringes grow fainter. They disappear, except in the peripheral part of the double plate, if the angle reaches zero¹⁰⁷ (Fig. 49b). The number and width of the fringes

¹⁰⁶ F. Savart, discussed by J. C. Poggendorf in *Ann. Physik Chem.*, **49**, 292 (1840).

¹⁰⁷ For details of the theory of the Savart plate, see J. Müller, *Ann. Physik Chem.*, **35**, 261 (1835).

depend on the double refraction and on the thickness of the plates. Savart plates of quartz are usually 4–5 mm. thick, and plates of the more birefringent calcite, 2 mm. or less.

The Savart plate is mounted in front of the analyzer so that the planes of the principal sections form an angle of 45° with the direction of transmission of the analyzer. This combination is the "Savart polariscope." If the polarizer or the Savart polariscope is rotated through 360° , four zero positions are met in which the fringes disappear in the center of the optical field. In each of these positions the fringes will reappear upon introduction of an optically active substance, and α is measured by rotating the polarizer or the Savart polariscope until the fringes disappear.

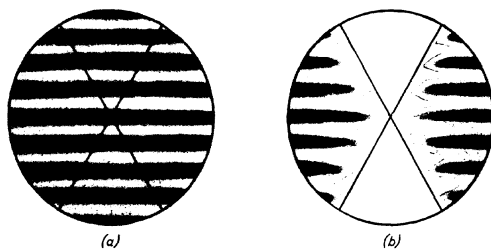


Fig. 49.—Aspect of optical field in Savart polariscope.

a, polariscope off zero position; *b*, in zero position. Crossed straight lines are reference lines.

The Savart polariscope has disadvantages: (1) It is not possible to distinguish a positive from a negative rotation, but the sign may be determined by the extinction method (page 1534) after removal of the Savart plate. (2) Rotations of α , $(90^\circ + \alpha)$, and $n(90^\circ + \alpha)$ cannot be distinguished. On the other hand, the polariscope has certain advantages: The brightness of the optical field is high in the zero position, where V_0 and the direction of transmission of the analyzer form an angle of 45° . More important is the very high sensitivity of the fringes toward small deviations from the zero position. Even the fairly crude procedure used by Wild in his original polaristrobometer¹⁰⁸ makes possible an accuracy of $1'$. The commercial polaristrobometers manufactured in France in the last century gave an accuracy of $0.5'$. An Andreas cross (two hairs crossed under an angle $\approx 90^\circ$) was used as a reference mark for the recess of the fringes toward the periphery upon approach of the zero position (Fig. 49b). Ac-

¹⁰⁸ H. Wild, *Ann. chim. phys.*, **3**, 501 (1864); *Ann. Physik Chem.*, **122**, 626 (1864).

according to Danjon¹⁰⁹ and Santon,¹¹⁰ the method can be improved so that it exceeds the best half-shade methods in sensitivity.

Perucca's Fringe Method.—A perfectly flat, very thin, slightly tapered glass plate, such as a microscope cover glass, exhibits in transmitted

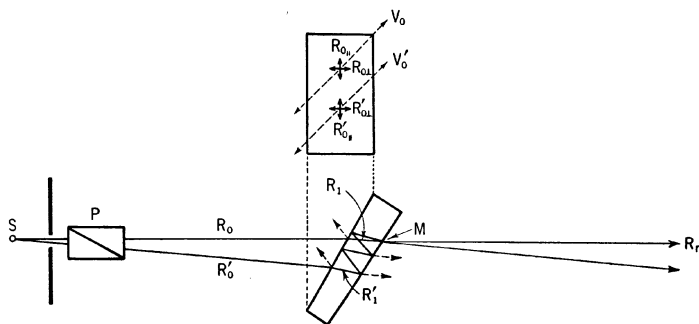


Fig. 50.—Perucca's fringe method.

Symbols \parallel and \perp refer here to direction of vibration with respect to plane of incidence.

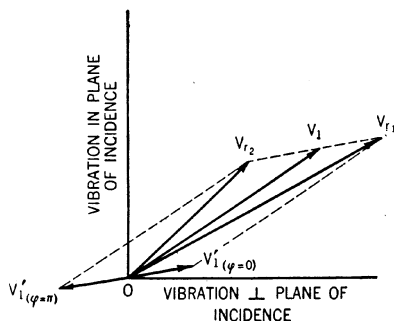


Fig. 51.—Principle of Perucca method.

Directions of vibration of R_r : $\varphi = 0$, phase difference between V_1' and V_1 is zero, 2π , or an even multiple of 2π ; $\varphi = \pi$, phase difference is π or an uneven multiple of π .

natural monochromatic light a series of straight equidistant dark fringes, caused by interference. The phenomenon is somewhat less simple with

¹⁰⁹ A. Danjon, *Rev. optique*, 5, 55 (1926).

¹¹⁰ L. Santon, *Rev. optique*, 7, 487 (1928).

incident linearly polarized light. The latter was used by Perucca,¹¹¹ for an interesting polarimetric method which is claimed to be as sensitive as that of Lippich.

The principle of the Perucca method is as follows. If the direction of vibration of the divergent incident coherent light of a point source, S , forms an angle of 45° with the plane of incidence (Fig. 50), the reflected component, $R_{0||}$, vibrating in the plane of incidence, is weaker than the orthogonal component, $R_{0\perp}$ (see page 1520). The amplitudes of the refracted components are therefore different and, according to figure 13, the result of their interference will be a rotation of R_1 with respect to R_0 . The same applies to R'_1 with respect to R'_0 . The degree of rotation is obviously different for R_1 and R'_1 , which intersect at M . In figure 51, the directions and relative amplitudes of vibration, V_1 and V'_1 , of R_1 and R'_1 , respectively, are shown

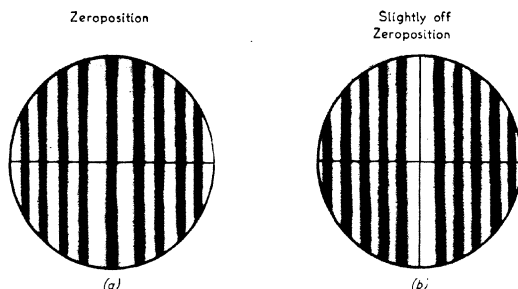


Fig. 52.—Optical field with Perucca method.

schematically. The result of interference between R_1 and R'_1 at M will be a linearly polarized ray, R_{r1} , defined by the vibration, V_{r1} , if the phase difference reached at M is $0, 2\pi$ or a multiple of 2π (subscript $\varphi = 0$). It will be a linearly polarized ray, R_{r2} , which has another direction of vibration, V_{r2} , if the phase difference is π or an uneven multiple of π (subscript $\varphi = \pi$). It will be an elliptically polarized ray for all other phase differences, except for $\pi/2$ or an uneven multiple of $\pi/2$ which cause circularly polarized light (page 1513). Upon introducing an analyzer, dark fringes will therefore be observed when the direction of transmission of the analyzer is perpendicular to either R_{r1} or R_{r2} . Assuming that extinction has been established for R_{r1} in the center of the plate, a rotation of the analyzer by 2ϵ will lead to the disappearance of the central fringe and to the appearance of two fringes symmetrical to the vanished central fringe. These fringes correspond to an extinction of R_{r2} . The phenomenon is shown schematically in figure 52. The observation of the "splitting" of the fringe into two fringes is facilitated by a reference line (vertical line in Fig. 52). The Perucca method is of practical interest, because any perfectly flat, slightly tapered, microscope cover glass may be used. The sensitivity of the method requires verification.

¹¹¹ E. Perucca, *Nuovo cimento*, 17, 1 (1940).

D. COMPLETE INSTRUMENT

Figure 53 shows the complete polarimeter of the type manufactured by *Gaertner Scientific Corp.*, Chicago, Ill. It is, like all commercial polarimeters, a half-shade apparatus. A Lippich prism of the triple field type is used as the polarizer. The field of the polarizer is observed through a telescope in front of the analyzer. The half-shade angle is varied by a vertical

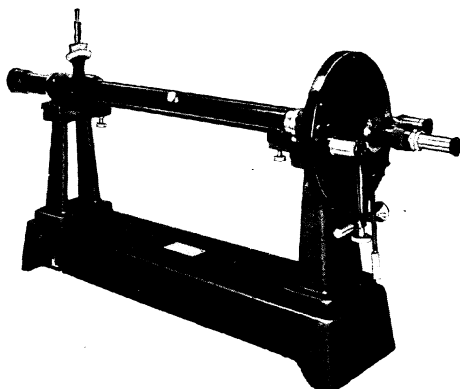


Fig. 53.—Gaertner Lippich triple field polarimeter (courtesy Gaertner Scientific Corp.).

lever on top of the polarizer mounting (left-hand side in Fig. 53). The graduated circle, allowing readings of 0.01° , is covered except for two small sections 180° apart from each other on which the readings are made on two verniers through magnifying glasses.

4. Visual Polarimetry with Compensator in Monochromatic Light

The angle, α , can be measured not only by a rotation of the analyzer or polarizer, but also by compensation with a second optically active substance of known rotatory power of a sign opposite to that of the substance investigated. The layer thickness of this compensator is varied while polarizer and analyzer remain crossed. Generally, quartz is used for compensation. The use of an optically active liquid in a cell of variable thickness is possible, in principle, but is not practical. Since a 1-mm. layer of quartz causes, at 20°C. , a rotation of $\pm 21.7^\circ$ at 5893 \AA. and of $\pm 25.5^\circ$ at 5461 \AA. , a variation of less than 1 cm. in the thickness of the plate will compensate any rotation ordinarily occurring in liquids in the visible spectrum. The problem of varying the layer thickness has been solved in a simple

manner by the Soleil¹¹² double wedge. A plane-parallel plate of quartz cut normally to the optic axis is cut diagonally into two wedges, and the ends of the wedges are ground off (Fig. 54). By shifting one movable wedge, Q_1^* , laterally, while keeping the other, Q_1 , in position, the rotation of the compensator is varied between a greatest and least value. The narrower the diaphragm, D , in front of the wedge, the further the movable wedge can be shifted in either direction. In order to obtain a zero rotation, a plane-parallel plate of quartz of opposite sign of rotation is added to the double wedge. In figure 54, the double wedge consists of levorotatory quartz, and the plate of dextrorotatory quartz. By giving to the latter

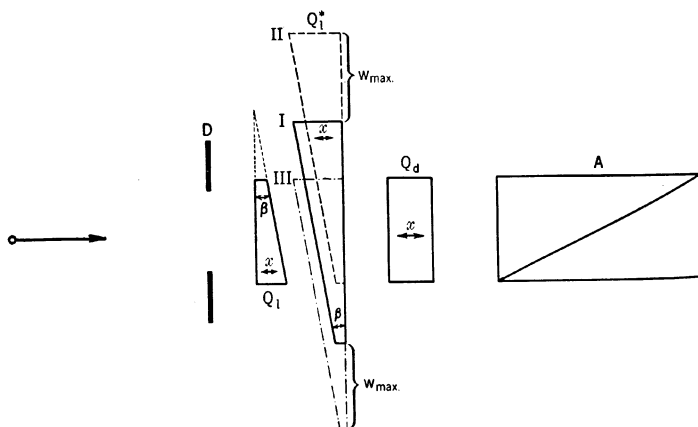


Fig. 54.—Standard quartz-wedge compensator.

I, zero position; II, compensation of negative rotation; III, compensation of positive rotation.

the thickness of the double wedge in position I, the problem of compensating both positive and negative rotations is also solved. Compensation of a positive rotation with the compensator of figure 54 requires a shift downward to position III of the movable wedge. In position II, a negative rotation is compensated.

The rotation produced by the compensator is proportional to the lateral displacement, w , of the movable wedge, w being zero in position I. Implicitly, the displacement of the movable wedge required for compensating the rotation of a third substance is proportional to this rotation. The

¹¹² H. Soleil, *Compt. rend.*, 21, 426 (1845).

range of compensation increases with both w and β . The largest rotatory power, $\alpha_{\max.}$, that can be compensated is therefore defined by:

$$\pm \alpha_{\max.} = \pm \alpha_Q w_{\max.} \tan \beta \quad (19)$$

where α_Q is the rotation of quartz per unit length. On the other hand, the sensitivity of the compensator and the accuracy of compensation increase for a given Δw with decreasing β . The choice of β should therefore depend on the accuracy and the range of compensation which are required. For both high accuracy and a wide range, a very long movable wedge and a small compensator angle β should be used. The range of commercial instruments generally does not exceed 150° and may be considerably

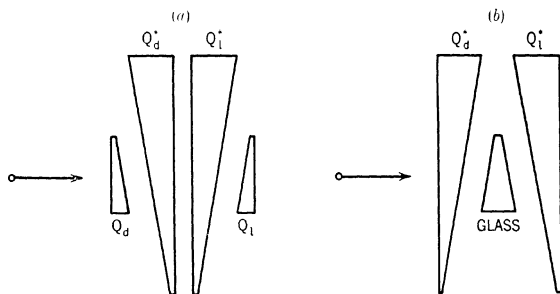


Fig. 55.—Improved quartz-wedge compensators.

a, Schmidt & Haensch compensator; *b*, Martens compensator;
 Q^* , movable quartz wedges.

smaller. A slight disadvantage of the Soleil quartz wedge is a systematic error introduced in the measurements if the slanting face of the movable wedge is not completely planar throughout its entire length. This error has been brought under control by a construction used in some polarimeters made by *Schmidt & Haensch* (shown in Fig. 55a). Both wedges, Q^* , can be displaced laterally; the others are stationary. The advantage of this construction is that a simultaneous displacement of both movable wedges in the same direction does not alter the zero position. One can therefore repeat a measurement by using other sections of the movable wedges and taking the mean value. The advantages of this procedure are great if the rotations to be compensated are small.

The movement of the movable wedge, or wedges, by means of rack and pinion, is recorded on a linear, usually horizontal, graduated scale. This, in commercial instruments, is mounted above the analyzer, close to the telescope. The scale may be divided in degrees and fractions of degrees

permitting the direct reading of the compensated rotation, or it may be divided in fractions of 100, or marked in other units, *e. g.*, give directly the concentration of sucrose.

There are a few other types of compensators which are different in detail but not in principle. Figure 55*b* shows, as an example, a Martens compensator. It consists of two movable wedges and of one immobile glass wedge.¹¹³ Polarimeters provided with compensators are mostly employed for sugar analysis. They are then called saccharimeters and are generally used in white light (see following section). Precision instruments are operated on the half-shade principle. The fringe principle could be used just as well. Figure 56 shows a saccharimeter manufactured by *Bausch & Lomb*.

The data obtained with a polarimeter without compensator are independent of temperature except for the temperature effect exhibited by the optically active substance in the polarimeter tube (page 1605). A polarimeter with quartz-wedge compensator, however, is sensitive toward temperature changes. The variation with the temperature, T (in degrees C.), of the optical rotation of quartz is given for the D line by:

$$\alpha_T = \alpha_{20} + \alpha_{20} \times 0.000143(T - 20)$$

where α_{20} stands for the optical rotation at 20° C. This correction factor is valid in the neighborhood of 20° C. It should be applied to the readings on the compensator scale—on the assumption that the latter had been calibrated for 20°—if a high precision in measurements of large rotations is desired. If, for example, the rotation measured at 25° C. is 100°, the deviation of the actual rotation from that read on the compensator scale amounts to 7'.

5. Visual Polarimetry in White Light

Polarimetry in white light has the advantage over polarimetry in monochromatic light in that the light flux in the polarimeter is incomparably larger with a given light source. This makes possible the use of very small half-shade angles, and consequently a high accuracy in the measurements defined by an error possibly as small as 2-3". However, the use of white light is limited to polarimeters with compensator and to the investigation of few substances; and even then the results have limited significance, although their practical value may be high.

The limitation of the use of white light to polarimeters with a compensator and to a few substances is due to the fact that the rotatory power varies with the wave length. Instead of extinction or field equality, respectively, a colored field or difference in coloring in the two half-fields would be obtained in a polarimeter without a compensator. However, even the

¹¹³ F. Martens, *Z. Instrumentenk.*, 20, 82 (1900).

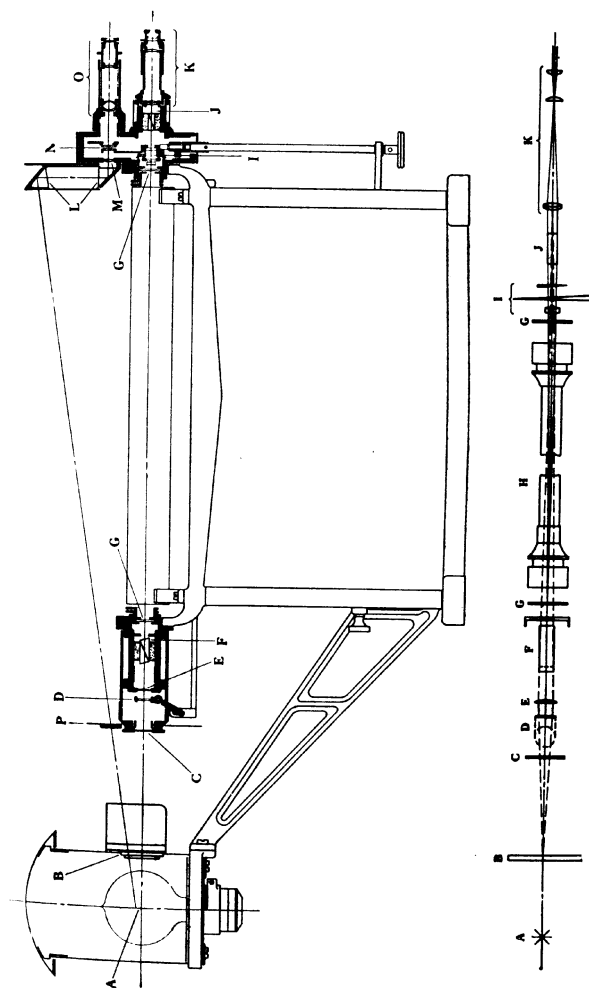


Fig. 56.—Bausch & Lomb saccharimeter (courtesy Bausch & Lomb).

- A, Light source.
 B, Ground-glass lamp window.
 C, Cover glass.
 D, Glass filter replacing dichromate cell.
 E, Condensing lens.
 F, Polariser.
 G, Splash glass.
 H, Observation tube.
 I, Quartz compensator.
 J, Analyzer.
 K, Observing telescope.
 L, Scale illuminating prism.
 M, Condensing lens.
 N, Scale and vernier plate.
 O, Scale-reading eyepiece.
 P, Glass filter in scale illuminating system.
 Q, Photometric field as seen from eye point.

use of a compensator is limited, because its rotatory power obviously must have the same dispersion as the investigated substance. By a fortunate coincidence, the rotatory dispersion of quartz is so close to that of several sugars, including cane sugar, that quartz compensators and white light can be used in the polarimetry of such sugars.

Table VI gives the variation of the rotatory power with wave length for several substances. The optical rotations of all substances are arbitrarily set equal to unity at wave length 6867 Å. Turpentine is apparently another of the few substances which could be investigated in white light using a quartz compensator. The table shows, on the other hand, that the dispersions of quartz and sugar are similar but not identical. The difference in the dispersions increases toward the violet. Polarimetry of sug-

TABLE VI
DATA OF LANDOLT^a FOR RELATIVE ROTATORY DISPERSION OF SEVERAL SUBSTANCES

Substance	Wave length, Å.					
	6867	6563	5890-5896	5270	4862	4308
Quartz	1	1.09	1.38	1.75	2.08	2.70
Sucrose	1	1.11	1.40	1.78	2.13	2.77
Turpentine	1	1.09	1.36	1.71	2.03	2.60
Cholesterol	1	1.24	1.53	1.93	2.36	3.02

^a H. Landolt, *Das optische Drehungsvermögen organischer Substanzen*. Vieweg, Braunschweig, 1898, p. 133.

ars in white light ("saccharimetry") must therefore be restricted to sugar solutions which do not exhibit large rotations. In other words, the solution must not be too concentrated, the approximate limit being a concentration of 40%. Since the differences in dispersion are most pronounced in the blue and violet, one can improve the measurements and somewhat extend the range of usable sugar concentrations by employing a light filter of potassium dichromate solution, which eliminates the region of short wave lengths (page 1528). For precision measurements, it is advisable to have such a filter even in the case of small rotations. In some commercial instruments (Fig. 56), a colored glass filter is used as a substitute for the liquid filter.

The significance of results obtained in white light is limited by the fact that they represent the "mean" of rotations, the statistical weight of which varies with the wave length. Factors affecting the significance of the "mean value" are the spectral sensitivity of the eye, the spectral distribution of the light source, and the dispersion of the rotatory power. Polarimetric measurements in white light have, therefore, mainly an analytical value in determining the concentration of substances of known rotatory power.

The polarizer and the analyzer must be suitable for white light. Any prism of the Glan-Thompson type, including compound prisms such as the Lippich or Jellett-Cornu, may be used. The polarization is uniform and complete for all wave lengths within the visible spectrum if the prisms are properly constructed and installed. Theoretically not as perfect, but practically satisfactory, are polarizers based on the principle of reflection. Since the refractive index of a reflector varies with the wave length, Brewster's angle (Eq. 11, page 1520) is dependent on the wave length, although very slightly. On the other hand, polarizers based on dichroism are unsuitable because of the variation of their polarizing power with the wave length.

The Laurent prism is somewhat deficient, since a quartz plate can be a half-wave plate for one wave length only (page 1544). Consider, for example, case *c* of figure 40. The field part of the analyzer covered by the quartz plate would be completely dark if the vibration emerging from the quartz plate were linear. Light of the wave length for which the plate is actually a half-wave plate would be extinct, but light of any other of the neighboring wave lengths would be transmitted to some extent due to ellipticity. Consequently, the respective half-field would appear slightly colored in white light. On the other hand, it has been shown (pages 1544 *et seq.*) that the position of field equality (Figs. 40*b* and 42*b*) is not affected. The zero position is therefore independent of the wave length, but the coloring of one of the fields, near the zero position, makes it difficult to locate the latter.

Far more disturbing is the color effect produced by those half-shade devices in which one half-field is covered by a plate of quartz cut perpendicular to the optic axis. Here, the coloring is due to the rotatory dispersion (see page 1501). At 6563 Å., the rotation produced by 1 mm. of quartz is 17.31°. At 4308 Å., it is 42.59°, *i. e.*, almost 2.5 times larger. Consequently, there is no definite zero position. Much more favorable is the situation if a biquartz plate is used (Fig. 43, page 1545).

We shall consider the case in which the largest rotation of the quartz—in the violet—is smaller than 90°. In figure 57, a thickness of 1 mm. is assumed for both the right and left quartz. The vibration, V_0 , incident upon the biquartz, is parallel to the direction of extinction, OE . The shaded area represents the range of rotations between 4500 and 6500 Å. as produced by the left quartz; the symmetrical blank area in the lower quadrant refers to the right quartz. It follows from the figure, *i. e.*, from the projections onto the OT axis, that field equality—the case considered—is characterized by a bluish tint of equal shade and brightness in both half-fields. Upon rotating V_0 by a small angle, *e. g.*, by introducing an optically active substance, the tint will change to whitish in one half-field and become fuller in the other. It will change to a saturated color as soon as the shaded area overlaps OE . This type of half-shade is useful, but less sensitive than the following.

We assume that the rotation of both halves of the quartz plate exceeds 90° (-90°), but is smaller than 180° (-180°) in the violet (layer thickness > 2 mm. and < 4 mm.). If the nicols are crossed, the biquartz shows an equal hue in both half-fields. The tinge is dominated by the wave length for which the rotation is exactly 90° . The optical field is very bright because of the transmission of light of all wave lengths. Rotation of the analyzer by 90° makes the aspect of the field far more favorable physiologically (Fig. 58). No light is transmitted of that wave length for which the rotation amounts to 90° and the two equally bright and equally colored half-fields have a saturated pure color which, upon a slight rotation of the

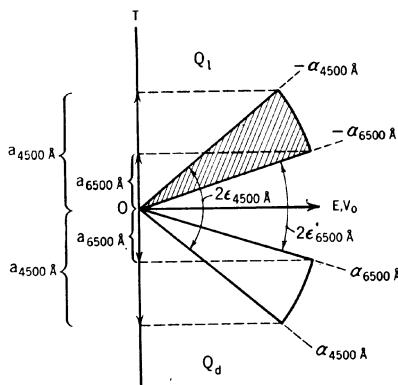


Fig. 57.—Functioning of thin biquartz as half-shade device in white light. Both Q_d and Q_1 are 1.0 mm. thick.

analyzer, undergoes a very pronounced antibat change in the two half-fields. The resulting contrast between the two half-fields is most strongly felt if the radiations which, in the position of field equality, are extinct or weakly transmitted belong to the yellow-green region (see Fig. 31, page 1532). Field equality is then characterized by a purple, so-called "sensitive" tint. A minute rotation leads to a reddish color in one half-field and to a bluish color in the other. The biquartz operated on the principle of the sensitive tint and known as the Soleil biquartz¹¹⁴ was, for a long time, the favored half-shade for saccharimeters. It is usually 3.75 mm. thick. The rotation is then 90° at $\sim 5500 \text{ \AA}$. and 82° at the D line. The uncertainty in finding the position of field equality amounts to a few minutes. This is satisfactory if the effects to be measured are 30° or more. The

¹¹⁴ H. Soleil, *Compt. rend.*, 20, 1805 (1845); 24, 973 (1847); 26, 162 (1848).

Soleil biquartz is unsuitable, however, for precision measurements and for measurements of small effects. It is worth while recalling that field equality is reached when the nicols are *parallel*, while usually in half-shade methods field equality indicates that polarizer and analyzer are crossed or nearly crossed.

Fringe devices may also be used for polarimetry in white light, but no elaborate attempts have been made in this promising direction. Probably

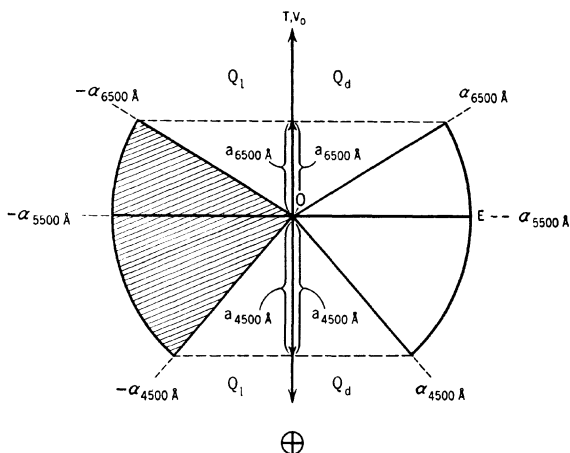


Fig. 58.—Soleil biquartz for polarimetry in white light. Both Q_d and Q_l are 3.75 mm. thick.

the first to realize this possibility was Wild,¹⁰⁸ who achieved with his polaristrobometer an accuracy of $2'$ in the determination of the zero position in the absence of an optically active substance, which is better than possible with the Soleil biquartz. The Senarmont arrangement also appears promising (see pages 1546 *et seq.*).

6. Visual Spectropolarimetry

A. SUCCESSIVE MEASUREMENTS IN MONOCHROMATIC LIGHT

Investigation of the dispersion of rotatory power is the purpose of spectropolarimetry. The technically simplest procedure determines the rotatory power successively for different monochromatic radiations, by means of any of the methods described above. However, this procedure is not spectropolarimetry in the strict sense, although the results obtained are equivalent to those obtained with a spectropolarimetric method.

B. PRINCIPLE OF FIZEAU AND FOUCAULT

The most frequently used spectropolarimetric principle was introduced by Fizeau and Foucault.^{115, 116} If an optically active material is inserted between parallel nicols, light of that wave length will be extinct, λ_E , for which the rotation amounts to 90° (Fig. 58). If the nicols are crossed,

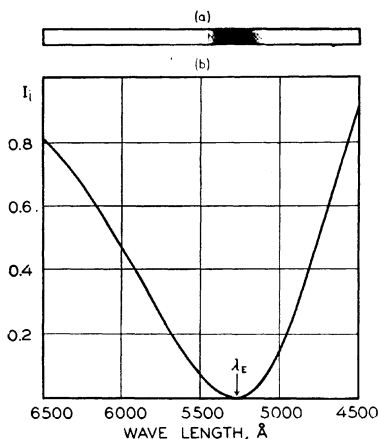


Fig. 59.—Fizeau-Foucault fringe observed between crossed nicols in continuous spectrum of white light with optically active substance—6.55-mm. layer of quartz—in the light path.

(a) aspect of fringe; (b) intensity distribution in spectrum.

light of that wave length will be missing for which the rotation amounts to 180° (see page 1560). The light emerging from the analyzer is therefore colored (pages 1559 *et seq.*); and if it is sent through a dispersing prism the spectrum obtained exhibits a dark fringe which is centered at the wave length at which the rotation is 90° or 180° , respectively. Figure 59a illustrates the latter case and figure 60a shows the rotation itself, both times for a plate of dextrorotatory quartz 6.55 mm. thick. Between crossed nicols, wave length 5269 Å. is extinct, the rotation being 180° . By means of equation (13) (page 1523), one obtains the intensity distribution in the light transmitted through the analyzer. This is shown by figure 59b. For the sake of simplicity, the brightness of the light source is assumed to

¹¹⁵ H. Fizeau and L. Foucault, *Compt. rend.*, 21, 1155 (1845).

¹¹⁶ O. J. Broch, *Repertorium der Physik (Dove's)*, 7, 113 (1846); *Ann. chim. phys.*, 34, 119 (1852).

be independent of the wave length. For physiological reasons (see page 1535), the fringe appears sharper and narrower the brighter the spectral region of maximum transmission. The fringe will shift if the analyzer or the polarizer is rotated, the direction of the shift depending on the sign of the rotatory power. In the case of a dextrorotatory substance, the fringe moves toward shorter wave lengths if the analyzer is rotated clockwise and vice versa.

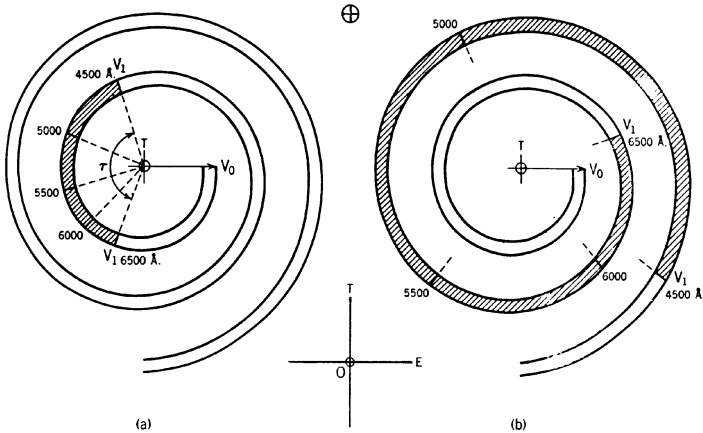


Fig. 60.—Variation in rotation of quartz with the wave length.

(a) the d quartz is 6.55 mm. thick, and the wave length extinct between crossed nicols is 5269 Å.; (b) the d quartz is 19.65 mm. thick, and the wave lengths extinct between crossed nicols are 6370, 5269, and 4600 Å.

The sign of the optical rotation is therefore determined from the direction in which the fringe is shifted. The magnitude of the rotation is found by one of the following methods:

(a) By determining the wave length, λ_E , at the center of the fringe while the nicols remain crossed (or parallel).

(b) By rotating the analyzer until the center of the fringe coincides with a reference mark corresponding to a known wave length within a continuous spectrum.

The easiest reference marks are those provided by the Fraunhofer lines if sunlight is used. They are located at 6867, 6563, 5893, 5270, 5175, 4861, 4308, and 4102 Å., disregarding the lines at the extreme ends of the visible spectrum. These lines were used by Fizeau and Foucault and by Broch.^{115,116} Other possibilities are the cali-

bration of the spectrum obtained from white light of an artificial light source by means of either the Fraunhofer lines¹¹⁷ or a line spectrum.¹¹⁸

(c) By rotating the analyzer until some line of a line spectrum (*e. g.*, of Hg) is completely extinct. In case *a*, the rotation, α_{λ_E} , is $\pm 180^\circ (\pm 90^\circ)$; in *b* and *c*, it is $\pm 180^\circ \pm \rho_A (\pm 90^\circ \pm \rho_A)$, ρ_A being the rotation of the analyzer. The dispersion of the rotatory power can be determined by bringing the center of the fringe successively into coincidence with several wave lengths.

Lippich Modification.—The procedure outlined above is not suitable for precise measurements because the fringe is too broad for coordination to a definite wave length. It can be made precise by means of a half-shade arrangement.¹¹⁹ If an analyzer with half-shade device is used, a spectrum is obtained which is divided into two parts by the dividing line

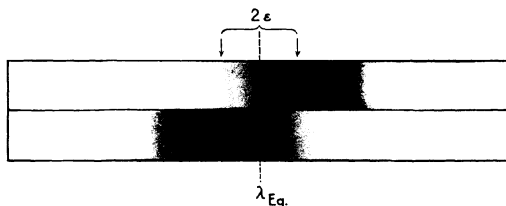


Fig. 61.—Lippich double fringe, in continuous spectrum of white light. $\lambda_{Eq.}$, wave length of "field equality."

of the half-shade. Both spectra are equally dim between crossed nicols in the absence of an optically active substance. In its presence, each of the two spectra exhibits a dark fringe. The centers of the two fringes are displaced with respect to each other (Fig. 61). The optical rotations at the wave lengths in the center of the two fringes differ by the half-shade angle, 2ϵ . The difference between the two wave lengths, in terms of \AA ., depends on the dispersion of the rotatory power of the material investigated. If, for example, with quartz and a half-shade angle of 2° , the center of one fringe is located at 5000 \AA ., the center of the fringe in the juxtaposed spectrum will be located at $5150 (4850) \text{ \AA}$.

Unless the half-shade angle is very large, the opposite tails of the two fringes will be on top of each other, as shown in figure 61. It is therefore

¹¹⁷ J. Stephan, *Sitzber. Akad. Wiss. Wien, Math.-naturw. Klasse, Abt. IIa*, 50, 88 (1864).

¹¹⁸ V. von Lang, *Ann. Physik Chem.*, **156**, 422 (1875).

¹¹⁹ F. Lippich, *Sitzber. Akad. Wiss. Wien, Math.-naturw. Klasse, Abt. IIa*, 91, 1070 (1885); **85**, 307 (1882).

easy to determine the wave length, $\lambda_{\text{Eq.}}$, at which the two juxtaposed fields have equal brightness. This wave length is obviously equidistant from the centers of the two fringes if the half-shade is symmetrical (page 1542). If the nicols are crossed, the rotation for $\lambda_{\text{Eq.}}$ is 180° . If the analyzer has been rotated by the angle ρ_A , the rotation at $\lambda_{\text{Eq.}}$ is $180^\circ + \rho_A$ or $180^\circ - \rho_A$, respectively. No $\lambda_{\text{Eq.}}$ will be found in the spectrum if the optical rotation in the visible region is smaller than 180° . A large rotation of the analyzer will then be necessary in order to have $\lambda_{\text{Eq.}}$ enter the visible part of the spectrum.

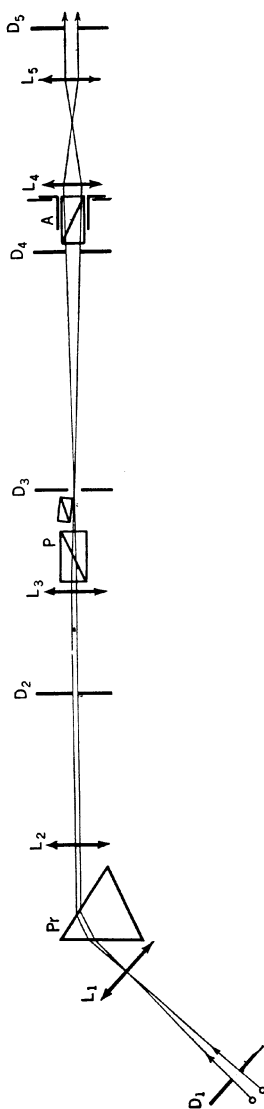
A wave length and a rotation can be coordinated with great precision by the half-shade method, provided that one determines $\lambda_{\text{Eq.}}$ accurately. The technique for doing so depends on whether a line spectrum or a continuous spectrum is used. In the former case, the analyzer is rotated until one line is equally bright in both spectra. This line represents $\lambda_{\text{Eq.}}$. (Upon rotating the analyzer further, by $+\epsilon$ or $-\epsilon$, the line will become extinct in the upper or lower spectrum, respectively.) The optical rotation can be determined for all spectral lines, in a series of successive measurements. If a source with continuous spectrum is used, a monochromator is generally combined with the polarimeter. For a given monochromator setting, the analyzer, or polarizer, is rotated until the two spectral strips, isolated by the monochromator, show equal brightness; or the analyzer and polarizer positions may be kept constant and the monochromator setting changed systematically. In the earlier techniques, the light traversed first the analyzer and then the monochromator, but the large amount of light passing through the polarimeter, only to be eliminated by the monochromator and therefore useless for the measurements, may be the source of stray light and impair the measurements.¹²⁰ It is therefore preferable to place the monochromator in front of the polarizer. The latter is then provided with the half-shade and a simple analyzing prism is used. Figure 62¹²¹ shows the scheme of a spectropolarimeter of this type, but with the polarimeter tube omitted.

The monochromator extends from D_1 to D_2 , the polarimeter from L_3 to A , and the telescope for observation of the dividing line of P from L_4 to D_6 .

A double or triple field Lippich prism is generally used. Less advantageous is the Soleil biquartz (page 1560) favored in earlier techniques. The precision of visual spectropolarimetric measurements depends, of course, not only on the factors discussed above, but also on the brightness of the two spectral strips (see the Weber-Fechner law, pp. 1534 *et seq.*), on the

¹²⁰ D. D. Brace, *Phil. Mag.*, 5, 161 (1903).

¹²¹ G. Bruhat, *Traité de Polarimétrie*. Editions de la Revue d'Optique théorique et instrumentale, Paris, 1930, p. 132.

Fig. 62.—Spectropolarimeter.¹²¹

half-shade angle, and on the dispersion of the prism or the monochromator. The smaller the half-shade angle and the higher the dispersion, the higher is the accuracy. In order to permit the use of a small half-shade angle and of a high dispersion, the brightness of the light source should be as high as possible and the direction of transmission of the polarizer should be crossed with the exit slit of the monochromator (page 1521).

Pachmanabhan¹²² claims that the accuracy of the Lippich method can be increased by adding an optically active quartz plate in the light path, because this reduces the range of wave lengths within which the two spectra have equal brightness. It seems questionable, however, whether this improvement is real. For the weight of the uncertainty, $\Delta\lambda$, is correspondingly increased, if $\Delta\lambda$ has the same value in both the old and the revised Lippich techniques.

Application to Large Rotations.—The optical rotation of true liquids and solutions rarely exceeds 180° in the visible. It may be larger in colloidal solutions and considerably larger in mesomorphic liquids (liquid crystals). In the latter instance, a 1-mm. layer may have a rotation of 10^4 or even 10^5 degrees (page 1587).¹²³⁻¹²⁵ Thick crystals of organic compounds, *e. g.*, those of camphor derivatives, may also have fairly large rotations. A simple polarimeter cannot be used in such cases unless the layer thick-

¹²² R. Pachmanabhan, *J. Indian Chem. Soc.*, **12**, 559 (1935).

¹²³ F. Stumpf, *Physik. Z.*, **11**, 780 (1910); *Ann. Physik*, **37**, 351 (1912).

¹²⁴ P. Gaubert, *Compt. rend.*, **164**, 405 (1917).

¹²⁵ G. Friedel, *Ann. phys.*, **18**, 273 (1922).

ness, or the concentration, is varied systematically, a tedious and time-consuming procedure. With a half-shade polarimeter, for example, one cannot distinguish between rotations of α , $180^\circ + \alpha$, and $n(180^\circ + \alpha)$. This difficulty does not arise with a polarimeter with compensator provided the quartz layers and angle β are sufficiently large. But then the absolute error becomes great (see page 1555). For large rotations, the spectropolarimetric technique therefore appears to be more suitable.

We assume that a continuous spectrum is used. The case of a line spectrum is discussed on pages 1568 and 1582. In figure 60a, the quartz plate was 6.55 mm. thick and the range of rotations, τ , between 6500 and 4500

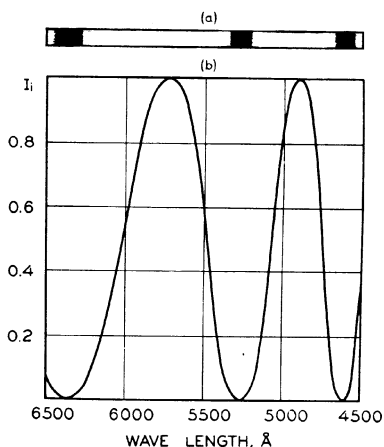


Fig. 63.—Multifringe spectrum between crossed nicols with rotations $> 360^\circ$.
(a) aspect of spectrum; (b) intensity distribution in spectrum. 19.65-mm. layer of quartz.

Å. was 136.8° ($115.2^\circ \rightarrow 252.0^\circ$). For a plate three times as thick (Fig. 60b), τ' is correspondingly three times as large as τ , and the range of wave lengths corresponding to τ becomes correspondingly smaller. As a consequence, the fringe centered at 5269 Å. now corresponds to a rotation of 540° instead of 180° and appears between one-half and one-third as broad as before. Moreover, the fringe is not the only one in the visible spectrum: an additional, narrower fringe is observed at 4600 Å. (rotation, 720°) and a second additional, broader fringe at 6370 Å. (rotation, 360°). The centers of these fringes are equidistant in terms of the rotations which they represent, the mutual distance being 180° , but not in terms of linear

distance. If the spectrum were a normal spectrum obtained by means of a grating, the spacing of the fringes would decrease strongly toward the ultraviolet, because of the strong increase of the rotatory power toward shorter wave lengths. This case is illustrated in figure 63.

It is also assumed, for the sake of simplicity, that the brightness of the light source is independent of the wave length and that the source emits a continuous spectrum. The λ_E are 6370, 5269, and 4600 Å

The decrease of the spacing toward the ultraviolet is counteracted if the spectrum is obtained by means of a prism because a prismatic spectrum is dilated toward the shorter wave lengths. The linear fringe spacings will therefore be the result of two antagonistic tendencies.

As the rotatory power increases, more and narrower fringes will enter the spectrum from the ultraviolet, while less than the equal number of fringes leaves the spectrum toward the infrared. Except for the stratified aspect of the spectrum (*spectre cannelé*), the situation is the same as in figure 63. The technique of coordinating a fringe to a wave length is the same as for a single fringe. The only difference is that one may dispense with the half-shade because the small width of the fringes makes the relative error in the coordination of a wave length to a fringe negligibly small. A simple spectropolarimeter for this case has been proposed by Maisel.¹²⁶

Since the rotation increases toward the violet end of the spectrum by 180° from fringe to fringe, it is easy to determine the absolute rotation for any wave length provided the multiple of 180° is known which is coordinated with any one of the fringes. This is found either (a) with a simple polarimeter on a layer of known thickness and thin enough to give a rotation of less than 180° , or (b) with the spectropolarimeter, by determining the number and position of the fringes for a somewhat thinner or thicker layer of the substance. Procedure b is preferable if the rotation is so large that it becomes difficult to secure a layer thin enough for a. In the case of liquid crystals, for example, a layer rotating less than 180° may be much smaller than one micron.

It is noteworthy that a simple polarimetric measurement may permit a very accurate determination of the film thickness of an optically active mesomorphic substance adsorbed at a transparent plate. A film thickness of less than 10 Å. may be measurable. Even in less extreme cases, the optical rotation may be used to determine the thickness of layers. A quartz plate, for instance, may be measured visually, with an error smaller than $\pm 0.5 \mu$, by using a half-shade polarimeter of average quality ($\lambda \sim 5000$ Å.). A still higher accuracy could be achieved in the ultraviolet.

The advantage of the spectropolarimetric measurement of large rota-

¹²⁶ S. Maisel, *J. Russ. Phys.-Chem. Ges.*, **42**, 277 (1910).

tions becomes still more apparent if the dispersion of the rotatory power is not normal, as assumed thus far, but anomalous. When the analyzer (or polarizer) is rotated, the fringes wander across the spectrum (see page 1568). If the dispersion of the rotatory power is normal, the direction of the shift is the same for all fringes and only the linear spacings may vary, growing larger or smaller depending on the direction of rotation of the analyzer and on the part of the spectrum considered. If the dispersion of the rotatory power is anomalous, *i. e.*, if the rotation is positive in one part of the visible

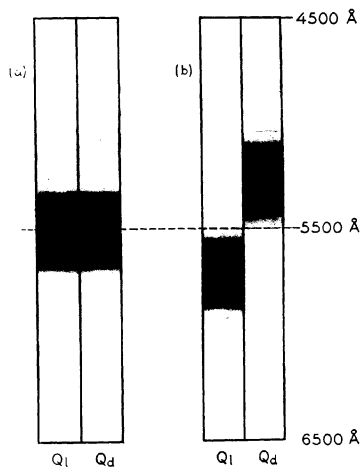


Fig. 64.—Fringes in continuous spectrum after passage of light through Soleil biquartz between crossed nicols: (a) in the absence and (b) in the presence of a levorotatory substance. (Dextrorotatory substance would displace fringes in inversed opposite directions.) See also figures 43 and 58.

spectrum and negative in another, then a rotation of the analyzer or polarizer will cause one set of fringes to move toward longer waves and another to move toward shorter waves. This provides an easy test for the character of the dispersion. Whenever the dispersion is anomalous, two neighboring fringes, which move in opposite direction, may differ by a rotation other than 180° . With this in mind, dispersion curves can be traced without error. For a still more elegant method of distinguishing between normal and anomalous dispersion, see page 1585.¹²⁷

¹²⁷ The importance of Fizeau-Foucault fringes for a quick survey of the character of dispersion of optical rotations has rightfully been stressed by J. Rabinovitch, *Rev. optique* 17, 161 (1938).

Wiedemann's Modification.—Wiedemann,¹²⁸ in an interesting modification of the method of Fizeau and Foucault, uses a modified Soleil biquartz. It may be recalled (see page 1560) that between parallel nicols both half-fields of the Soleil biquartz appear uniformly colored in white light, and that a rotation of analyzer or polarizer or the introduction of an optically active substance causes an antibat color change of the two half-fields (see Fig. 58, page 1561).

If the light emerging from the analyzer is sent through a dispersing prism, the two spectra obtained are divided by the dividing line of the biquartz; if the nicols are parallel, a continuous dark fringe crosses both spectra. It is centered in both spectra, at the wave length for which the rotation by the

quartz is $\pm 90^\circ$ (Fig. 64a). This is a characteristic difference from the Lippich modification discussed above. If the analyzer or polarizer is rotated or an optically active substance is introduced, the fringes evidently move in opposite directions (Fig. 64b). The rotation of an optically active substance can therefore be measured by rotating the analyzer or polarizer until the fringes unite again into a single band. This is the spec-

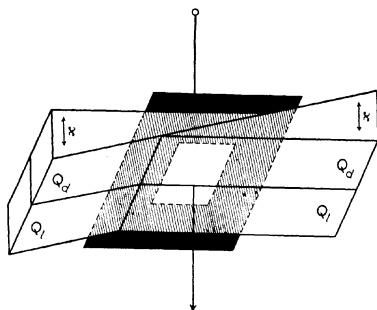


Fig. 65.—Wiedemann's biquartz of variable thickness.

tropolarimetric analogue to the polarimetric re-establishment of color equality in the two half-fields.

By varying the thickness of the Soleil double wedge the zero position of the two adjacent fringes (rotation of $\pm 90^\circ$), i.e., the spectral coincidence of the fringe centers in both spectra, can be obtained for any wave length. Such an increase in versatility of the simple Soleil biquartz, used with originally parallel nicols and in connection with a dispersing prism, was accomplished by Wiedemann. The construction of the device follows from Figure 65.

C. LOMMEL'S SPECTROPOLARIMETRIC METHOD

A quartz plate cut parallel to the optic axis is birefringent toward light of normal incidence (page 1542). This leads to color phenomena if a sufficiently thick quartz plate is used in white light between two nicols (page 1559). If the optic axis forms an angle of 45° with the incident linear vi-

¹²⁸ G. Wiedemann, *Die Lehre von der Elektrizität*. Vieweg, Braunschweig, 1893-1898.

bration and the nicols are parallel, then light of that wave length is extinct for which the phase difference amounts to 180° or to an uneven multiple of 180° . If the nicols are crossed, the same wave length is most strongly transmitted. If the phase difference is 360° or a multiple of 360° , extinction takes place with crossed nicols and maximum transmission with parallel nicols. The quartz plate has no effect if the angle between the optic axis of the plate and the direction of the incident vibration is 0° or 90° .

We now send white light, emerging from the analyzer, through a dispersing prism, the angle between the optic axis of the plate and the direction of the incident vibration being 45° . Then each wave length for which the phase difference is 180° or an uneven multiple of 180° will be the center of a dark fringe on a bright background if the nicols are parallel, and the center of a bright region of maximum transmission on a less bright background if the nicols are crossed. The conditions for obtaining a dark fringe or a region of maximum transmission, respectively, are reversed if the phase difference is 360° or a multiple of 360° . The spectrum is therefore traversed by a large series of dark fringes if the quartz plate is thick enough ($\gg 0.5$ mm.). The appearance of the spectrum is then similar to that traversed by Fizeau-Foucault fringes

in figure 63, except that this time the large number of narrow fringes gives a hatched aspect to the spectrum. However, if the Soleil or Wiedemann biquartz is rotated around the direction of light propagation as the axis, the Fizeau-Foucault fringes remain unaffected, whereas the fringes under discussion here disappear when the optic axis of the plate forms an angle of 0° or 90° with the incident vibration. The whole spectrum will then be extinct for crossed nicols or bright for parallel nicols.

If an optically active substance is introduced in front of the quartz plate between crossed nicols, the spectrum remains stratified except for a narrow bright band centered at the wave length for which the optical rotation, α , is 45° (Fig. 66). An increase in the optical rotation moves the bright band to another wave length and an equally large but inverse rotation of the polarizer returns it to its original position. The rotation can therefore be determined for any wave length by rotating the polarizer until the center of the band coincides with the respective wave length. For this wave length, the optical rotation is $45^\circ + \rho_P$ or $45^\circ - \rho_P$, respectively, depending on the direction of the rotation, ρ_P , of the polarizer.

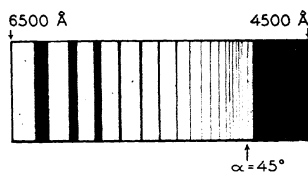


Fig. 66.—Appearance of continuous spectrum with thick birefringent quartz plate (cut parallel to optic axis) and optically active substance between crossed nicols.

If there are many fringes in the spectrum, the bright band should stand out well. This would require a very thick quartz plate. It appears that no method has been based on this simple principle. Lommel's related method¹²⁹ requires less quartz. A quartz wedge, *Q*, is used (Fig. 67). A prismatic effect of the quartz wedge is avoided by adding a glass wedge, *G*. The spectral resolution is carried out perpendicular to the slope of the wedges, and the fringes are therefore strongly curved (Fig. 68). The fringes at border *AB* of the spectrum correspond to those obtained with a thick quartz plate, and the less numerous fringes at border *CD*, to those with a thinner plate. The bright fringe is well defined due to the obliquity of the fringes.

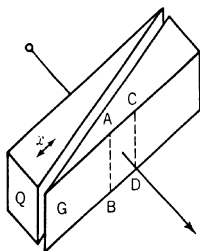


Fig. 67.—Lommel's wedge combination.

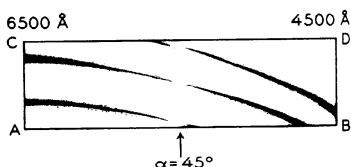


Fig. 68.—Appearance of continuous spectrum with Lommel's wedge combination and optically active substance between crossed nicols.

Lommel's method could be improved for precision measurements by using two quartz wedges with the optic axes forming a small angle. The two juxtaposed spectra obtained can easily be visualized. The zero position and α could be found by determining the wave length of brightness equality in the two spectra in analogy to Lippich's technique (page 1564). Such a modification of Lommel's method might be promising for opaque substances.

D. OTHER SPECTROPOLARIMETRIC PRINCIPLES

The two spectropolarimetric principles described above, those of Fizeau and Foucault, and of Lommel, are not the only ones. An important further principle, that of Hussell and Nutting, will be discussed later (on page 1583) because it has comparatively little significance for visual spectropolarimetry, except in the case of very large rotations. Some modifications of the Fizeau-Foucault method, which also have comparatively little significance for visual spectropolarimetry, will also be discussed in the section on nonvisual spectropolarimetry.

¹²⁹ E. Lommel, *Ann. Physik Chem.*, **36**, 731 (1889).

7. Nonvisual Polarimetry

A. METHODS

Visual methods are generally limited to the visible region of the spectrum. Though they can be extended into the ultraviolet and infrared by means of fluorescence or phosphorescence effects (pages 1603 and 1604), no precision is possible in such measurements. For precision work in the ultraviolet and in the infrared, nonvisual methods are used. On the other hand, the nonvisual methods are not restricted to the ultraviolet and infrared, but may be of advantage in the visible spectrum. There are essentially three principles of nonvisual polarimetric technique: photographic, photoelectric, and thermoelectric.

Photographic Technique.—The photographic plate can be used within the entire ultraviolet region, in the visible region and in the near infrared approximately up to 12,000 Å. For most of the visible, the far ultraviolet, and the infrared, sensitized plates are necessary, as manufactured, for example, by *Eastman Kodak Co.* In the far ultraviolet—below 2800 Å. and especially below 2000 Å., where light absorption by gelatin is strong—photosensitive strata free from or low in gelatin (Schumann plates¹³⁰) or ordinary photographic plates covered with a film of fluorescent oil^{131,132} are used.

The main disadvantage of the photographic technique is the time-consuming operations involved in it. If the compilation of α is based on photometric or microphotometric analysis of the plate (see, *e. g.*, page 1582), a photometer or microphotometer is necessary. However, the photographic method has important advantages over other methods. The effect on a photographic plate is dependent not only on the intensity of illumination, but to a certain degree also on the duration of exposure.

According to the empirical law of Schwarzschild:¹³³

$$K = It^p \quad (20)$$

where K is the optical density of the exposed and developed plate, I is the illumination, t is the time of exposure, and p is an empirical constant. Depending on the value of I , p varies between ~ 0.9 and ~ 1.2 . Long exposure may therefore give useful results in the case of low brightnesses.

Another important advantage of the photographic technique is the possibility of obtaining a polarimetric record which extends over a large

¹³⁰ See, *e. g.*, E. C. C. Baly, *Spectroscopy*. Vol. II, Longmans, Green, London, 1927, p. 380.

¹³¹ G. R. Harrison, *J. Optical Soc. Am.*, **11**, 113 (1925).

¹³² J. Duclaux and P. Jeantet, *J. phys. radium*, **2**, 156 (1921).

¹³³ K. Schwarzschild, *Astrophys. J.*, **11**, 89 (1900).

spectral region. Furthermore, the photographic method is the most sensitive nonvisual one after the photon counter method (page 1534). Finally, the photographic record is the only convenient means of analyzing rotations in excess of 360° , whether inside or outside the visible region.¹³⁴

Photoelectric Technique.—The photoelectric technique is by far the most convenient nonvisual technique. Primarily for this reason photoelectric polarimetry can be expected to be of increasing importance. There are, however, disadvantages: (a) Any photoelectric cell has, like the eye, a maximum sensitivity within a certain generally narrow spectral region. Depending on the type of the cell used, the maximum sensitivity may be in the visible or in the ultraviolet. A small fraction of the infrared may also be covered by phototubes. The wave length of 12,000 Å. is at present the approximate limit of useful response of photocells. The spectral region within which a simple photoelectric polarimeter with one photocell can be used is therefore far more limited than the region available to photographic polarimetry. (b) If the dispersion of rotatory power is to be studied, a series of successive measurements in monochromatic light or a scanning of the spectrum is necessary. This also is a disadvantage as compared with photographic methods. (c) A photoelectric instrument is not ready for instant use if it contains an amplification system. The tubes must first be "warmed up" for a period of possibly as long as 15 minutes before precision measurements can begin. (d) A disadvantage of photoelectric methods compared with visual methods is the necessity of introducing a several-stage external or internal amplification in order to approach the accuracy of visual measurements (see page 1588). The latter type of amplification is of particular interest because of its comparative simplicity. It is realized in the electron multiplier (photon multiplier).¹³⁵

Thermoelectric and Bolometric Techniques.—In the far infrared, neither photographic nor photoelectric methods can be used. Measurements must be based on thermoelectric or bolometric principles.¹³⁶

¹³⁴ For further information on photographic technique, see J. Strong, *Procedures in Experimental Physics*, Prentice-Hall, New York, 1938, pp. 449–493, and on photographic theory, C. E. K. Mees, *The Theory of the Photographic Process*, Macmillan, New York, 1942.

¹³⁵ For detailed information on the theory and use of photoelectric tubes, see R. H. Muller, R. L. Garman, and M. E. Droz, *Experimental Electronics*, Prentice-Hall, New York, 1942; J. Strong, *op. cit.*, pp. 396–449; K. Henney, *Electron Tubes in Industry*, McGraw-Hill, New York, 1937; A. L. Albert, *Fundamental Electronics and Vacuum Tubes*, Macmillan, New York, 1938. For an elementary introduction to the subject see, E. P. Anderson, *Audel's Electronic Devices and Their Application*, Theo. Audel & Co., New York, 1942.

¹³⁶ See J. Strong, *op. cit.*, pp. 305–340.

B. POLARIMETER ACCESSORIES FOR THE ULTRAVIOLET

On its path through an ordinary polarimeter, the light traverses the following substances: glass, calcite, Canada balsam, and air. Each of these substances, while transparent in the visible, becomes opaque in the ultraviolet. The absorption of Canada balsam, often used for cementing polarizing prisms, begins at 3400 to 2800 Å., depending on the brand. It is, therefore, advisable to use air as an interprismatic medium below 3500 Å. (Glan prism, Fig. 21, see page 1514), or, if the polarimetric measurements are not extended below 2400 Å., glycerol. Still further in the ultraviolet, calcite also begins to absorb light strongly. A calcite polarizing prism of standard length is practically opaque slightly below 2400 Å. Quartz prisms or fluorite prisms must then be used. The limit of transmission of pure samples of the former is approximately 1850 Å., and that of the latter 1200 to 1000 Å., depending on the purity of the crystal. Because of the comparatively weak double refraction of both quartz and fluorite, it is difficult to obtain satisfactory polarizing prisms of the Glan type. Double image prisms are therefore used.¹³⁷ If, finally, measurements are to be carried out below ~ 1000 Å., polarization by reflection (see page 1520) is the only possible method. Glass lenses (flint or crown) used in front of the polarizer or behind the analyzer, and dispersing prisms made of glass, are unsuitable below 3500–3800 Å., depending on the thickness. Special ultraviolet-transmitting glasses may be used, however, as far down as 2700–3000 Å. Special phosphate glasses¹³⁸ may have an appreciable transmis-

TABLE VII
APPROXIMATE LIMITS OF USEFUL LIGHT TRANSMISSION THROUGH VARIOUS SUBSTANCES

Material	Approximate shortest wave length, Å.
Glass disks, of commercial polarimeter tubes, 2–3 mm. thick.....	3650
Film of Canada balsam in nicols.....	3400–2800
Microscope cover glass, 0.05 mm. thick.....	2650
Calcite; 4-cm. layer (= average length of polarizer + analyzer combined).....	2400
Film of glycerol in nicol if used instead of Canada balsam.....	2300
Quartz, ~ 2 -cm. layer.....	1850
Fluorite, ~ 2 -cm. layer (pure crystals).....	1000

sion at even shorter wave lengths. Plastics are often particularly unsuitable in the near ultraviolet. Most satisfactory are quartz and fluorite.

¹³⁷ See, for example, W. Kuhn, *Ber.*, **62**, 1727 (1929).

¹³⁸ See, for example, W. W. Coblenz and R. Stair, *J. Research Natl. Bur. Standards*, **13**, 773 (1934).

A brief enumeration of comparative transparencies is given in tables VII and VIII.

The limiting wave length given in table VII for the glass disks holds on the assumption that a powerful light source, *e. g.*, a Pointolite lamp (page 1529) is used. The range of wave lengths given for Canada balsam signifies that the limiting wave length depends on the brand. The mercury line with the shortest wave length transmitted perceptibly from a mercury arc through a microscope cover glass of the specified thickness is 2654 Å. The light transmission through calcite of the specified length is, at the specified limiting wave length, 20%; and it is already as small as 0.9% at the still shorter wave length of 2310 Å. Very short nicol prisms may be used down to 2200 Å., however, if the time of photographic exposure is long enough.¹³⁹ The light transmission of fluorite of the specified length is as high as 80% at 1860 Å.

TABLE VIII

TRANSMISSION OF SPECIAL ULTRAVIOLET GLASSES AND COMPARISON WITH COMMON GLASSES AND SYNTHETICS^a

Wave length, Å.	Per cent transmission through 10-mm. layer						
	Correx	Vita	Uviol	Hard crown	Pyrex	Dense flint	Poly- styrene
3303	87	81	86	65	18	10	.
3087	78	56	35	6
2749	12

^a Data from B. K. Johnson, *Proc. Phys. Soc. London*, **55**, 291 (1943).

In the far ultraviolet where fluorite becomes opaque, lenses must be replaced by concave aluminized or silvered mirrors, and dispersing prisms by gratings. It may be added that the use of glass for the cover glasses of polarimeter tubes is a less serious matter, because they may be made very thin. Ordinary microscope cover glasses (about 0.05 mm. thick) transmit sufficient light above approximately 2700 Å. At still shorter wave lengths, however, special glasses, quartz, fused silica, fluorite, or cellophane windows must be used. Fluorite has the disadvantage of being very fragile in thin layers. If quartz is used (see Table IX), one of the disks must be *d*-quartz, the other *l*-quartz of exactly the same thickness in order to eliminate optical rotation. The light absorption by air becomes an important factor at wave lengths below 1800 Å. Vacuum polarimeters must be used for the extreme ultraviolet.

As regards the light sources for ultraviolet polarimetry, again the choice is between sources of a continuous spectrum and sources of a line spectrum.

¹³⁹ S. Landau, *Physik. Z.*, **9**, 417 (1908).

The most preferred of the former type is the hydrogen discharge tube obtainable from *Central Scientific Co.*, Chicago, Ill. The Nernst lamp (see following section) may be used down to approximately 2800 Å. Among the light sources with a line spectrum, the mercury and the iron arcs are favored. The wave lengths of the most intense spectral lines of the mercury arc between 4100 and 2500 Å. are: 4046.8; 3663.3; 3650.2; 3125.6–3131.8; 3023.5; 2536.5. Less common, but most interesting because of their very high intrinsic brightness, are sparks such as cadmium, copper, or aluminum sparks¹⁴⁰; their use is restricted to photographic polarimetry.

Ultraviolet polarimetry is the most important branch of polarimetry for research work. Optically active absorption bands of organic substances are generally in the far ultraviolet. Consequently, the rotatory power generally reaches very high values in the intermediate ultraviolet. A few comparative data are given in table IX. It is worth while adding that

TABLE IX
SPECIFIC OPTICAL ROTATIONS, $[\alpha]$, IN THE VISIBLE AND ULTRAVIOLET OF SELECTED ORGANIC COMPOUNDS IN WATER^a

Substance	Grams in 100 g. aqueous solution	Temperature, ° C.	$[\alpha]$ in degrees at wave length in Å.				
			6000	5000	4000	3000	2750
Sucrose	3.45	18	62.1	99.8	149.9	297.7	394.4
Lactose	15.81	19	51.9	64.6	110.1	258.3	310
Tartaric acid	28.625	..	9.4	7.5	-6.0	-166.0	-296.8
KSb tartrate	6.635	..	125	158	281	758	1020

^a Data from P. G. Nutting, *Phys. Rev.*, **17**, 1 (1903). $[\alpha]$ is defined on page 1606. Disks of polarimeter tubes were 2.872 mm. thick, one of *d*-quartz and the other of *l*-quartz (see page 1576).

water as solvent may be used down to at least 2500 Å, particularly if the photographic technique (long exposure) is used.

C. POLARIMETER ACCESSORIES FOR THE INFRARED

As in the case of ultraviolet polarimetry, one of the principal problems in infrared polarimetry is the transparency of the polarizing prisms. Calcite may be used up to approximately 20,000 Å. At still larger wave lengths, only polarization by reflection is suitable. For details see Chapter XXI.

¹⁴⁰ G. Joos, in W. Wien and F. Harms, *Handbuch der Experimentalphysik*. Vol. 21, Akadem. Verlagsgesellschaft, Leipzig, 1927.

One of the best sources of infrared radiation is the Nernst lamp. Operated at a temperature of 2400°K. , it radiates 75% of the radiation of a black body of the same temperature up to approximately $150,000\text{ \AA.}$ ¹⁴¹ Its major advantage is that the brightness is easily kept constant within 1%. Nernst lamps can be obtained from *Adam Hilger, Ltd.*, London, and are fairly easy to make.¹⁴² Another useful source of infrared radiation is the Welsbach mantle, known from the early periods of gas illumination; it is a mantlelike skeleton of interwoven fibers of rare earths, e. g., of 99.2% thorium oxide and 0.8% cerium oxide. When heated by a Bunsen burner, the emission of light is strong in the middle portion of the visible spectrum. It is very poor between 10,000 and $50,000\text{ \AA.}$, but rises rapidly toward larger wave lengths, reaching, at $150,000\text{ \AA.}$, 80% of the emission of a black body of equal temperature (1800°K.).¹⁴³ An important advantage of this light source is the large radiating surface area. Favored, because they are easy to operate, are tungsten lamps, particularly those containing a ribbon filament, or tungsten arcs (Pointolite lamps, see page 1529). If the bulb of such a lamp contains a quartz window, it can be used up to approximately $40,000\text{ \AA.}$

The light sources discussed above give a continuous spectrum from which monochromatic radiation may be obtained by means of appropriate dispersing prisms or gratings. In the far infrared, above $300,000\text{ \AA.}$, monochromatic radiation must be obtained by some other method. Generally favored is the method¹⁴⁴ of "residual rays."

Water has a series of very strong absorption bands in the near infrared which make the polarimetric investigation of aqueous solutions of optically active substances difficult or impossible above 9000 \AA. The molecular extinction coefficients are 0.46, 1.30, 30.5, and 104 for 9700, 12,000, 14,400, and $20,000\text{ \AA.}$, respectively. Carbon dioxide also has a series of absorption bands, so that it may be useful to conduct the measurements in air as free of carbon dioxide as possible.¹⁴⁵

¹⁴¹ For emission spectra of the Nernst lamp, see Chapter XXI, figure 36, and figures 125 to 128 in W. W. Coblenz, *Investigations of Infrared Spectra*, Carnegie Inst. Wash. Pub. No. 35, October, 1905.

¹⁴² H. D. Griffith, *Phil. Mag.*, **6**, 50, 263 (1925).

¹⁴³ For details, see H. Rubens, *Ann. Physik*, **18**, 725 (1905); *Physik. Z.*, **7**, 187 (1906).

¹⁴⁴ H. Rubens and E. F. Nichols, *Phys. Rev.*, **4**, 314 (1897).

¹⁴⁵ For experimental details on work in the infrared see: G. B. B. M. Sutherland, *Infrared and Raman Spectra*, Methuen, London, 1935; J. Lecomte, *La spectre infrarouge*, Presses Univ. France, Paris, 1928; C. Schaefer and F. Matossi, *Das ultrarote Spektrum*, Springer, 1930; W. W. Coblenz, *loc. cit.*, footnote 141; and R. B. Kurnes, R. C. Gore, U. Liddel, and Van Zandt Williams, *Infrared Spectroscopy*, Reinhold, New York, 1944.

8. Photographic Polarimetry and Spectropolarimetry

The main field of photographic polarimetry and spectropolarimetry is the ultraviolet, although photographic methods may be useful in the visible, particularly for spectropolarimetric measurements, and in the near-infrared.

A. PHOTOGRAPHIC POLARIMETRY IN MONOCHROMATIC LIGHT

Photographic polarimetry in monochromatic light may be carried out by adapting any of the visual methods. The main difference, except for substituting a photographic plate for the eye, and for the choice of the half-shade angle, is that visual methods generally, but not necessarily, call for the observation of a virtual image, *e. g.*, of the face of the half-shade, by means of a telescope, while a real image must be formed on the photographic plate. In the visible, photographic polarimetry with monochromatic light hardly has an advantage. In the ultraviolet or the near-infrared, photographic spectropolarimetry deserves preference over photographic polarimetry with monochromatic light unless the latter achieves a higher accuracy. This holds at present only for the methods of Bruhat and his collaborators.^{146,147} With either the extinction method or a half-shade method, a series of exposures is made on a single photographic plate for systematically varied analyzer positions. By making the images small enough and by shifting the plate after each exposure in the most economical manner, Bruhat and Pauthenier¹⁴⁷ obtained sixteen exposures on a single plate. The developed plate is analyzed by means of a microphotometer. The analyzer or polarizer position which corresponds to complete extinction or complete field equality, respectively, can be determined with an error which does not exceed 0.25'. In order to make the method sufficiently convenient, Bruhat *et al.* synchronized an automatic stepwise movement of the analyzer with an automatic stepwise shift in the position of the photographic plate.

The optimum half-shade angle in photographic polarimetry—and also in photographic spectropolarimetry—is 90°. This is a significant difference compared with visual polarimetry. The reasons are discussed in the section on photoelectric polarimetry (page 1592) where the situation is analogous.

B. PHOTOGRAPHIC SPECTROPOLARIMETRY

All the visual spectropolarimetric methods can be used in photographic spectropolarimetry. In addition, a few other methods based on principles

¹⁴⁶ G. Bruhat, *Ann. phys.*, **3**, 246 (1915).

¹⁴⁷ G. Bruhat and M. Pauthenier, *Rev. optique*, **6**, 163 (1927). This paper contains

a valuable review of photographic methods in the ultraviolet.

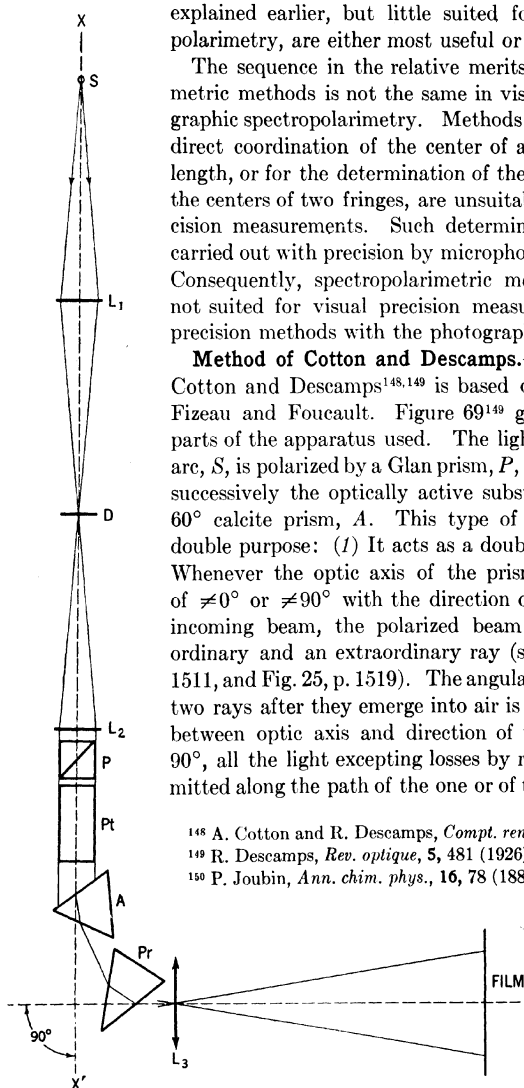


Fig. 69.—Photographic spectropolarimeter of Cotton and Descamps.¹⁴⁸

explained earlier, but little suited for visual spectropolarimetry, are either most useful or very promising.

The sequence in the relative merits of spectropolarimetric methods is not the same in visual and in photographic spectropolarimetry. Methods which call for the direct coordination of the center of a fringe to a wave length, or for the determination of the distance between the centers of two fringes, are unsuitable for visual precision measurements. Such determinations are easily carried out with precision by microphotometric analysis. Consequently, spectropolarimetric methods which are not suited for visual precision measurements may be precision methods with the photographic technique.

Method of Cotton and Descamps.—The method of Cotton and Descamps^{148,149} is based on the method of Fizeau and Foucault. Figure 69¹⁴⁹ gives the essential parts of the apparatus used. The light from a mercury arc, *S*, is polarized by a Glan prism, *P*, and then traverses successively the optically active substance in *Pt* and a 60° calcite prism, *A*. This type of prism¹⁵⁰ serves a double purpose: (1) It acts as a double image analyzer. Whenever the optic axis of the prism forms an angle of $\neq 0^\circ$ or $\neq 90^\circ$ with the direction of vibration of the incoming beam, the polarized beam is split into an ordinary and an extraordinary ray (see Fig. 18 III, p. 1511, and Fig. 25, p. 1519). The angular separation of the two rays after they emerge into air is 16° . If the angle between optic axis and direction of vibration is 0° or 90° , all the light excepting losses by reflection is transmitted along the path of the one or of the other ray, i. e.,

¹⁴⁸ A. Cotton and R. Descamps, *Compt. rend.*, **182**, 22 (1926).

¹⁴⁹ R. Descamps, *Rev. optique*, **5**, 481 (1926).

¹⁵⁰ P. Joubin, *Ann. chim. phys.*, **16**, 78 (1889).

rotation of either P or A leads alternately to extinction of the ordinary and the extraordinary ray. (2) Prism A acts as a dispersing prism, resolving each of the two rays into a mercury spectrum. A is followed by an additional dispersing prism, Pr , made of quartz, which is traversed by the ordinary ray only. A diaphragm prevents the less refracted extraordinary ray* from entering the quartz prism. The angle of the quartz prism is $47^\circ 14'$. The angle of incidence is such as to produce a total deviation of

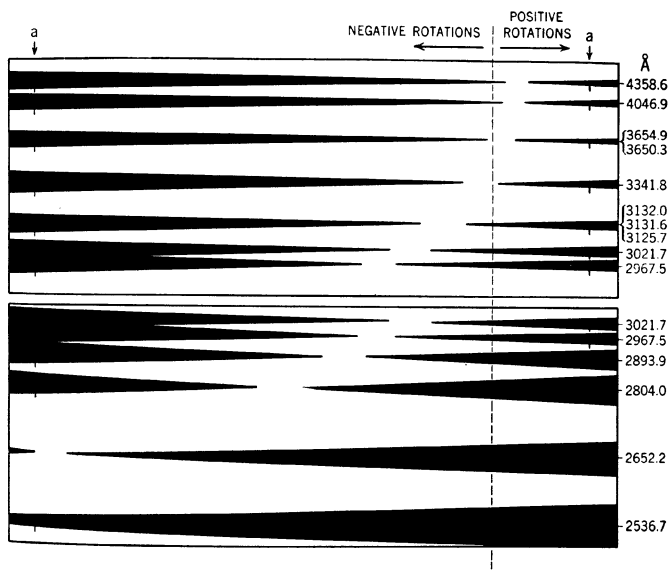


Fig. 70.—Schematic record of Hg line spectrum obtained at 23°C . with Cotton-Des-camps apparatus for 5.55-cm. layer of tartaric acid (4.951 g. in 100 cc. solution).¹⁴⁸ Height of wedge at given rotation represents relative halation and, implicitly, relative optical density.

nearly 90° with respect to the original direction of propagation of the polarized beam for the 3023 \AA . mercury line. If the spectrograph, consisting of the two prisms, A and Pr , and lens L_3 , is given a full rotation around the polarimeter axis, XX' , the brightness of the mercury spectrum varies twice between zero and a maximum. In the method of Cotton and Des-camps, the spectrograph does not undergo a full rotation, but oscillates slowly within a range of about 60° . By focusing the spectrum upon a stationary photographic film—which is curved around the XX' axis of the

* Not shown in figure 69.

polarimeter—elongated spectral lines are obtained on the photographic record, as shown schematically in figure 70. These lines are interrupted for that position of the spectrograph which brings about extinction of the ordinary ray. Microphotometric analysis of each line, along its length, gives very accurately the center of extinction. For the coordination of the centers of extinction to a rotation, ρ_{sp} , of the spectrograph, two calibrating exposures are made on the same film while the spectrograph is at rest in either of two extreme positions which are read on a stationary graduated circular scale. By using a wide spectrograph slit for the calibrating exposures, the two extreme positions of the spectrograph are recognized by the wider spectral lines, at α . The center of extinction is then easily coordinated to a certain rotation, ρ_{sp} . The optical rotation, α , is obtained for each spectral line by determining microphotometrically the displacement of the centers of extinction with respect to the “zero” record. In the latter, obtained in the absence of the optically active substance, the centers of extinction are all at the same height of the film, as indicated by the dotted line across the record.

Cotton and Descamps covered a spectral range extending from 4358 to 2536 Å. One centimeter on the photographic record corresponded to a rotation, ρ_{sp} , of 1° . Under this condition, the microphotometric record of the spectral lines made it possible to evaluate the center of extinction and, implicitly, α with an error not in excess of 0.02° .

Method of Landau.—Landau¹⁵¹ adapted the Lippich modification of the Fizeau-Foucault method (page 1564) to photographic spectropolarimetry. The two juxtaposed line spectra of an iron arc are photographed

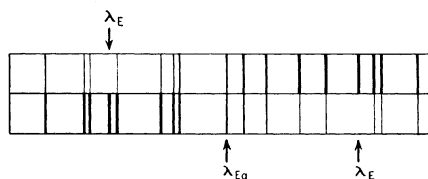


Fig. 71.—Spectral record of iron line spectrum obtained with Landau's method.

and the spectral line showing equal optical density in both spectra is determined photometrically; or, if necessary, the wave length is interpolated microphotometrically at which a spectral line would have equal optical density in both spectra. An important advantage of the iron arc is the abundance of spectral lines. For the same reason, the iron arc is unsuitable for visual spectropolarimetry. The method of Landau is illus-

¹⁵¹ S. Landau, *Physik. Z.*, **9**, 417 (1908).

trated schematically by figure 71. A decrease in optical density is indicated by decreasing the width of the spectral lines, as was done in figure 70.

The photographic record of one double spectrum gives only the rotation for one wave length if the rotations are smaller than 360° . In order to obtain the dispersion of rotatory power, a series of exposures is necessary. There is enough space on a photographic plate of standard size to accommodate eight to ten double spectra. Nevertheless, the necessity for successive exposures is a great disadvantage as compared with the method of Cotton and Descamps. On the other hand, a photographic spectropolarimeter operating on Landau's principle is easier to construct than the spectropolarimeter of Cotton and Descamps. The method has been modified and perfected by Darmon¹⁵² and by Lowry and collaborators,¹⁵³⁻¹⁵⁶ *e. g.*, by the introduction of a variable half-shade instead of the invariable one used by Landau, and by the use of a triple spectrum instead of the double spectrum. A triple field half-shade is employed for this purpose. Lowry and Coode-Adams¹⁵⁴ also used a continuous spectrum in the visible. The error in the determination of α has been reduced to approximately $10'$ (0.17°). The method has also been modified for spectropolarimetry in the far ultraviolet.¹⁵³⁻¹⁵⁷

The disadvantages of Landau's method disappear or become irrelevant if the rotations are larger than 360° . A single photographic record may then give the dispersion of rotatory power, because several spectral lines may show equal optical densities each in both spectra. All these lines differ by an angular distance of 180° (see page 1567). In addition, the absolute error of the method becomes generally unimportant for very large rotations, the relative error being almost negligible. In the case of very large rotations, Landau's spectropolarimetric photographic method is therefore most recommendable. It is then also most conducive for the visible region of the spectrum.

Method of Hussell and Nutting.—The double wedge of figure 44 (page 1547) between crossed nicols and traversed by monochromatic light is crossed by a dark fringe. If the wedge is thick enough, it is crossed by a series of parallel dark fringes, the centers of which differ by a rotation of 180° . The fringes are displaced upon introduction of an optically active substance into the light path (page 1547). In white light, colored fringes are observed due to the dispersion of the rotatory power. The color phenom-

¹⁵² E. Darmon, *Ann. chim. phys.*, **22**, 247, 495 (1911).

¹⁵³ T. M. Lowry, *Proc. Roy. Soc. London*, **A81**, 472 (1908); **A212**, 261 (1912).

¹⁵⁴ T. M. Lowry and W. R. C. Coode-Adams, *Proc. Roy. Soc. London*, **A226**, 391 (1927).

¹⁵⁵ T. M. Lowry and M. A. Vernon, *Proc. Roy. Soc. London*, **A119**, 706 (1928).

¹⁵⁶ T. M. Lowry and H. K. Gore, *Proc. Roy. Soc. London*, **A135**, 13 (1932).

¹⁵⁷ W. Kuhn, *Ber.*, **62**, 1727 (1929).

ena are shown schematically in figure 72 for the case of crossed nicols. Only the central fringe remains dark in the absence of an optically active substance. With a double wedge nearly three times as thick, spectral resolution gives a pattern on a photographic positive, as shown in figure 73. Only the dark central fringe is perpendicular to the length of the

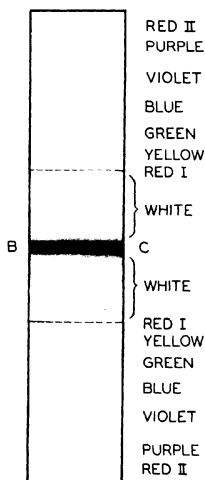


Fig. 72.—Appearance of optical field in white light with double wedge of figure 44 between crossed nicols.

Fringe is equivalent to zero fringe of a single quartz wedge, as considered in figure 75.

spectrum, while the other dark fringes are convergent toward the short wave lengths if the dispersion is normal. However, the mutual distances of the fringes, in terms of rotations, are the same— 180° —throughout the spectrum.

The fringes converge according to a parabolic function if the spectrum is normal and the rotatory power obeys Biot's law (pages 1496 and 1607). If the spectral region investigated is limited to 2000 \AA. or less, the fringes appear nearly linear. Figure 73 applies to these conditions. With a prismatic spectrum, the fringes converge according to a hyperbolic function.

If a line spectrum is used instead of a continuous spectrum, the various spectral lines will be intersected by nearly horizontal dark lines. The spectral strip between the dotted lines in figure 73 may aid in visualizing the appearance of such a spectral line. The introduction of an optically active substance results in a displacement of all fringes, including the central fringe, perpendicular to BC in figure 73. The displacement can be measured by means of a reference line (hair) which marks the original position, BC , of the central fringe. The shift gives the magnitude of the rotation, and the direction of displacement gives its sign (see page 1547).

This spectropolarimetric method was introduced by Hussell,^{158,159} who used a continuous spectrum (Fig. 73), and, making the fringes visible by phosphorescence, applied the method to infrared polarimetry (page 1604). Nutting¹⁶⁰ introduced the same principle later on in photographic spectropolarimetry, employing the line spectrum of a spark (Zn-Al alloy). Nutting employed a traveling microscope for measuring the shift of the fringes on the plate. No statement is made on the mag-

¹⁵⁸ A. Hussell, *Ann. Physik Chem.*, **43**, 498 (1891).

¹⁵⁹ R. S. Minor, *Ann. Physik*, **10**, 581 (1903).

¹⁶⁰ P. G. Nutting, *Phys. Rev.*, **17**, 1 (1903).

nitude of the error but the rotations are given in 0.1° , suggesting that the error did not exceed this value.

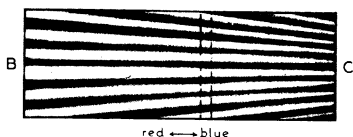


Fig. 73.—Spectropolarimetric record of Hessel-Nutting obtained by spectral resolution of the field in figure 72. Thicker quartz layer.

The method appears to be open to considerable improvement. By substituting a microphotometric analysis for the traveling microscope, the displacement could nowadays be measured more accurately so that the method could be used also for rotations of the order of magnitude of 10° . The mutual distance of the fringes and the displacement could be enlarged in future investigations by a cylindrical lens in the dispersing system. Finally, it appears unnecessary to have more than two symmetrical fringes in addition to the central fringe. Angle β of the double wedge in figure 44 could therefore be reduced, increasing the displacement and therefore the sensitivity and accuracy of the method. It is not impossible that such improvements would enable the method of Hessel and Nutting to compete in precision with the method of Cotton and Descamps. These two methods are, at present, the only photographic spectropolarimetric methods which give the dispersion of the rotatory power by means of a single exposure. Both methods afford a quick and easy survey of the character of the dispersion because the direction of the displacement reveals the sign of the rotatory power and its variation with the wave length.

Stumpff's Spectropolarimetric Method for Mesophases (Liquid Crystals).—A drop of an optically active mesophase on a glass slide will

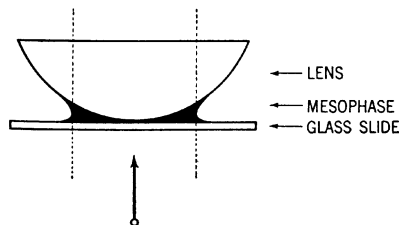


Fig. 74.—Lehmann's arrangement for measuring rotatory power of mesophases (liquid crystals).

spread to a circular wedge if a planoconvex lens is set into the drop (Fig. 74). A plane-parallel composite layer is thus obtained which is free from

optical distortion between the dotted lines of figure 74, if the refractive indices of the lens and of the mesophase are very similar. Between crossed nicols in monochromatic light, the circular wedge exhibits the circular analogue (Fig. 75b) to the fringes observed on a thick quartz wedge cut perpendicular to the optic axis (Fig. 75a). Each of the circular fringes represents the geometrical locus of a phase difference of $n \times 180^\circ$, n being 1 for the innermost fringe, 2 for the second, and n for the n th fringe. The center of the fringe pattern should be a dark spot if the glass slide and the center of the lens were in optical contact. The absence of such a dark

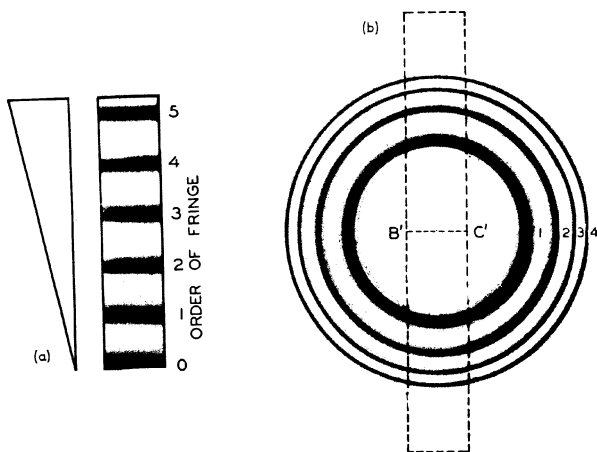


Fig. 75.—Fringes between crossed nicols in monochromatic light produced (a) by single quartz wedge, (b) by circular liquid wedge of figure 74.

spot in actual experiments indicates that there is no optical contact, but a film of mesophase between the center of the lens and the glass slide (see page 1568). Because of the curvature of the lens, the fringes are narrower the larger their diameter. From the known curvature of the lens and the distance between the centers of two adjacent fringes, the optical rotation per unit layer can be determined. This simple and very elegant method of measuring the rotatory power of mesophases by means of a polarizing microscope is described by Lehmann.¹⁶¹ By carrying out measurements successively at different wave lengths, the dispersion can be compiled.

Because of the great sensitivity of mesophases toward minute changes

¹⁶¹ O. Lehmann, *Ann. Physik*, 2, 649 (1900); 18, 796 (1905).

in temperature, Stumpf's^{162,163} spectropolarimetric modification of Lehmann's method for white light, which excludes the time element, is preferable. If a diaphragm restricts the passage of light to a rectangular section of the circular wedge between the dotted lines in figure 75b, spectral resolution will lead to a pattern similar to that of figure 73. The zero fringe at the half height, $B'C'$, of the spectrum is, of course, absent. Moreover, the pairs of symmetrical fringes now represent equal rotations of equal sign, while they represented equal rotations of opposite sign in the Hussell-Nutting pattern. Figure 76 is the photographic pattern obtained at 80° C. with the mesophase of pure cyanobenzylidene aminocinnamate, which shows an anomalous dispersion of rotatory power in the visible. The rotation, per millimeter layer, is, with -6000° , relatively "small" at 6420 Å., in excess of $-16,000^\circ$ at 6100 Å., in excess of $+30,000^\circ$ at 5200 Å. and $21,000$ – $26,000^\circ$ between 5080 and 4460 Å., with a minimum at 4800 Å. The optically active absorption band, responsible for the excessive rotations, reaches its peak between 5600 and 5800 Å. Within this region, the rotation goes through zero, as is to be expected from

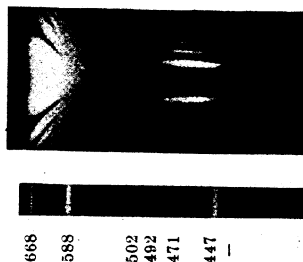


Fig. 76.—Photographic record¹⁶² of rotatory dispersion of a mesophase, as obtained by Stumpf's method with a continuous spectrum. λ in reference spectrum in millimicrons. Analysis of pattern shows changes in rotation with wave length from $> -16,000^\circ$ to $> +30,000^\circ$ with an error in evaluation of $\pm 25^\circ$. $B'C'$ runs horizontally.

figure 11 (page 1502). The narrow spectral region of excessive values of $d\alpha/d\lambda$ appears washed out on the pattern. The rotation is determined by measuring, for different wave lengths, the distance between the centers of two adjacent fringes. The smaller the distance, the larger is the value of α . As in the method of Hussell and Nutting, the dispersion of the rotatory power is obtained in a single exposure.

9. Photoelectric Polarimetry and Spectropolarimetry

Although photoelectric methods were introduced in polarimetry twenty years ago,¹⁶⁴ photoelectric polarimeters are still rarely used; methods in existence are subject to various improvements and additional methods

¹⁶² F. Stumpf, *Ann. Physik*, **37**, 351 (1912).

¹⁶³ F. Stumpf, *Physik. Z.*, **11**, 780 (1910).

¹⁶⁴ K. Mayrhofer, *Thesis*, Univ. of Würzburg, 1924. H. von Halban, *Nature*, **119**, 86 (1927).

are likely to be developed. The present section deals primarily with those already used and with their present state of development. A discussion going beyond these points may be found elsewhere.^{164a}

A. COUNTING LIGHT QUANTA VS. MEASURING PHOTOELECTRIC CURRENT

The best photosensitive layers used in commercial photocells for the ultraviolet liberate, on an average, 1 photoelectron per 1000 incident light quanta. The best photosensitive layers with optimum response in the visible require 15–50 quanta per photoelectron, *i. e.*, 3–10 times more than are necessary for the sensation of light with the visual purple of the human retina (page 1534). If the number of quanta per time unit is small, the electron output is nearly proportional to the incident light energy. Photon counters could therefore be used for a very sensitive polarimetric method suitable for, but restricted to, measuring rotations so small, $<0.5'$, that they cannot be measured by any other conceivable means. It would of course be necessary to use two counters, one registering the fluctuations in the quantum output of the light source. No literature is yet published on the use of photon counters in polarimetry.¹⁶⁵

In contradistinction to the photon counter, which counts single photoelectrons, the use of photoelectric cells is based on the measurement of the photoelectric current created by the emission of a sufficiently large number of photoelectrons per second. The minimum number of photoelectrons required per second for a steady current is approximately 100, *i. e.*, the minimum number of light quanta which must strike the photosensitive layer is 100,000 per second. This means, in practical terms, that only a fluctuating current is obtained if it is smaller than 10^{-16} amp. The photoelectric current can of course be amplified, but the data given show that the useful amplification is limited. The few comparable data which are available¹⁶⁶ seem to indicate that, even with the high amplification factor of 3.5×10^7 , the sensitivity of photoelectric determinations in the ultraviolet is still slightly inferior to the optimum sensitivity of visual methods in the visible (page 1556) and also inferior to the potential sensitivity of photographic methods (see page 1534). A high-quality photocell with optimum response in the visible should allow one to

^{164a} W. Heller, unpublished results.

¹⁶⁵ The reader interested in this possibility is referred to the following fundamental investigations on the use of the Geiger-Müller counter in photometry: M. Schein and M. L. Katz, *Nature*, **138**, 883 (1936); M. L. Katz, *Physik. Z. Sowjetunion*, **9**, 254 (1936); K. O. Kiepenheuer, *Z. Physik*, **107**, 145 (1937); B. Stoll, *Helv. Phys. Acta*, **8**, 3 (1935); K. H. Kreuchen, *Z. Physik*, **94**, 549 (1935), and **97**, 625 (1935); H. Geiger and W. Müller, *Physik. Z.*, **30**, 489 (1929).

¹⁶⁶ G. Bruhat and A. Guinier, *Rev. optique*, **12**, 396 (1933).

exceed the average precision of visual methods slightly if a current differential, $\Delta j = 10^{-15}$ amp., is made measurable. Persons with particularly sensitive, specially trained, and well-adapted eyes can be expected, however, to surpass any photoelectric registration, judging from recent visual photometric measurements of Russian authors.

B. PHOTOELECTRIC POLARIMETRY

Fundamentals.—A photocell can give the absolute value of a light intensity but it cannot make possible a comparison between two light intensities in a single operation. Just the opposite holds for the human eye. This means (a) that the absolute variation of I with σ , as expressed by figure 29 (page 1524), is of fundamental importance in photoelectric polarimetry, and (b) that true half-shade methods, as discussed in the section on visual polarimetry (pages 1535–1546), are not applicable here unless two photocells are used simultaneously.

Such a procedure is not free from objection. It is difficult to find two photocells with identical spectral sensitivity curves, so that the use of balanced photocells is generally possible for a single wave length only. In addition, the sensitivity curves may change with time and change unequally, due to changes in the photosensitive surface layer, particularly if the incident light intensities are not very small.

It follows from equation (13) (page 1523) that:

$$\frac{dI}{d\sigma} = I_t \frac{d(\sin^2 \sigma)}{d\sigma} \quad (21)$$

that is, a photoelectric polarimeter is most sensitive if $\sigma = 45^\circ$. Let us assume, for example, that $\alpha = 0.2^\circ$ and that it is measured according to the photoelectric method described on page 1598. The difference in the galvanometer deflections in the absence and presence of the optically active substance will be 300 times larger if $\sigma = 45^\circ$ instead of 0° . This holds, of course, only on the assumption that the galvanometer deflection, *i. e.*, the photoelectric current, is proportional to the intensity of light incident upon the photocell. This proportionality which can generally be realized except for high amplifications (page 1592) is assumed in this discussion.

The photoelectric current is not only dependent on the light intensity, *i. e.*, on the number of light quanta incident per unit time on the unit area, but also on the width of the beam, *i. e.*, on the total exposed area of the photosensitive layer. For a given light intensity, the photoelectric current can therefore be increased by selecting a wider aperture for the polarizing and analyzing prism. A particularly wide aperture at a reasonable price is possible with polarizing devices such as the Polaroid. The use of Polaroids in photoelectric polarimeters for routine technical measurements is therefore more justifiable than in visual or photographic polarimetry.

The photosensitive layer of a photocell is occasionally anisotropic, *i. e.*, the photoelectric current may vary with the direction of vibration of the incident linearly polarized light. It is therefore advisable to rotate not the analyzer but the polarizer. If the latter is not possible, the photocell should be rotated together with the analyzer.¹⁶⁷

A Cotton effect does not seriously affect visual measurements of α as long as the ellipticity due to it is small (page 1502). This does not hold if the measurement of α is based, as in some of the following photoelectric methods, on intensity measurements in successive experiments instead of intensity comparisons.

Photoelectric Equivalent of Visual Extinction Method.—An instrument which imitates the visual extinction method¹⁶⁸ cannot be used for accurate measurements, since $\sigma = 0^\circ$. If the rotations are very large, *i. e.*, in excess of 50° , the *relative* error may be small enough for routine experiments or for exploratory experiments.

This method, which is independent of light absorption or light scattering, is of course not restricted to the approximate determination of the analyzer position leading to extinction; it may also be used for determining the approximate position leading to maximum transmission, an alternative which is not possible in visual polarimetry.

Methods Related to Half-Shade Method.—*Method of Symmetrical Angles.*—Incomparably more accurate than the preceding method is the method of interpolation. From a number of measurements, carried out with systematically varied analyzer or polarizer positions, the position of complete extinction can be interpolated with great accuracy, as demonstrated by the analogous photographic method of Bruhat (page 1579). Particularly convenient and accurate and only possible in photoelectric polarimetry is the following simple interpolation method: Two analyzer positions, defined by the readings ω_{0a} and ω_{0b} on a graduated scale in the absence of the optically active substance and by ω_{1a} and ω_{1b} in the presence of the substance, are determined for which the galvanometer deflections are equal. Figure 77 illustrates primarily the first pair of experiments.

$$\omega_{E_0} = (\omega_{0b} + \omega_{0a})/2 = \omega_{0a} + \rho_0/2 \quad (22)$$

and consequently:

$$\alpha = \omega_{E_1} - \omega_{E_0} = (\omega_{1b} + \omega_{1a})/2 - (\omega_{0b} + \omega_{0a})/2 \quad (23)$$

ω_E being the interpolated reading which would correspond to extinction.

For maximum sensitivity, the galvanometer deflection found for $\sigma_0 = \pm 45^\circ$ is restored, by rotation of analyzer or polarizer, after the optically

¹⁶⁷ L. Ebert and G. Kortüm, *Z. physik. Chem.*, **B13**, 105 (1931).

¹⁶⁸ E. J. B. Willey, *J. Sci. Instruments*, **20**, 74 (1943).

active substance has been introduced. If, however, the latter strongly absorbs or scatters light, the restoration of the same deflection implies that $\sigma_1 \neq 45^\circ$ (it is $\sigma_0 + \Delta\sigma$ in Fig. 77). It may then be advisable to choose smaller deflections in the second pair of experiments.

In figure 77, the broken curve—valid in the presence of a light absorbing optically active substance—is made to coincide with the unbroken curve at E_0 . Actually, the former is displaced toward higher $\rho_{A(P)}$ values, as curve *B* in figure 83 (page 1597) is displaced with respect to curve *A*.

This method, which may be called the "method of symmetrical angles," was introduced by Kenyon and Dickes.¹⁶⁹ With an amplified photoelectric circuit, an accuracy of 0.01° was achieved in the red and of 0.1° in the violet.

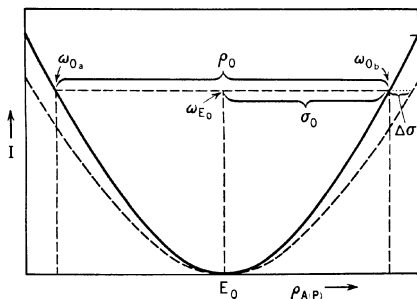


Fig. 77.—Method of symmetrical angles.

Quasi Half-Shade Methods.—Two light intensities recorded *simultaneously* are compared in a true half-shade method. This is not possible in photoelectric polarimetry unless two photocells are used. With one photocell, the closest approach to a half-shade method is the comparison of light intensities recorded in two *successive* experiments. The method of the symmetrical angles is therefore related to half-shade methods. The former requires four readings on a graduated circle instead of the two in a typical half-shade method. Still more closely related to the latter are, therefore, methods introduced by Bruhat and collaborators, which require only two readings on the scale for calculating α . These methods will be called "quasi half-shade methods."

In the method of Bruhat and Guinier,¹⁶⁶ a Glan-Thompson analyzer is preceded by a biquartz of the Soleil type (page 1560). Each of the two halves, Q_1 and Q_2 , is large enough to fill the optical field, and is brought

¹⁶⁹ J. Kenyon, *Nature*, 117, 304 (1926).

into the light path successively by lateral displacement (Fig. 78). The analyzer is rotated, first in the absence and then in the presence of the optically active substance, until the rapid lateral displacement of the biquartz is without effect upon the deflection of the galvanometer.

A series of stepwise rotations of the analyzer is necessary to find the exact position of "field equality" by this procedure. In order to accelerate the measurements, the biquartz is first removed, and the approximate position of extinction is established. After inserting the biquartz, only a small number of stepwise rotations is necessary in order to find the accurate position. The number of steps may be reduced further by interpolation (pages 1579 and 1590).

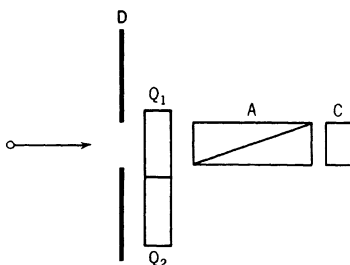


Fig. 78.—Quasi half-shade arrangement of Bruhat and Guinier.

The *absolute* difference in the light intensity transmitted through Q_1 and Q_2 is decisive for the sensitivity of the photoelectric quasi half-shade. It is thus clear from figure 34 (p. 1537) that the optimum half-shade angle is 90° (Fig. 34a). This is a fundamental difference compared with visual polarimetry, where the *relative* difference in light intensity is decisive, and where, consequently, the smallest physiologically advisable half-shade angle should be selected (Fig. 34b). This argument is valid on the assumption that the deflection of the galvanometer is proportional to I , a condition which could not be realized with the high amplification factor of 10^7 in the apparatus of Bruhat and Guinier. In order to reach the best sensitivity, three interchangeable biquartz plates were used, defined by half-shade angles of 41.5° , 16.6° , and 8.3° , respectively, for 4358 \AA .

The method of Bruhat and Guinier has recently been made still more sensitive¹⁷⁰ by introduction of a pentode instead of the triode used previously. A change in the photoelectric current of $7.5 \times 10^{-17} \text{ amp.}$, detected with a ballistic galvanometer, produced a deflection of 1 mm. Although

¹⁷⁰ G. Bruhat, A. Blanc-Lapierre, J. Schiltz, and G. Raoult, *Compt. rend.*, 214, 615 (1942).

this instrument was not used for measurements of rotations, it could evidently be used for this purpose. Bruhat emphasizes that an instrument operated with such an excessive amplification factor can only be used in the complete absence of extraneous vibrations and that it is very delicate also in other respects. It follows from the discussion on page 1588 that currents which are so small are strongly fluctuating.

An earlier, technically less simple, but interesting, quasi half-shade method is also described by Bruhat.¹⁷¹ A Wollaston prism, *W*, is used as the analyzer (Fig. 79). The two rays emerging from such a prism vibrate at right angles (page 1540). They thus have equal intensity only if the polarized beam, incident upon the prism, vibrates under an angle of 45° with respect to the optic axes, a condition fulfilled

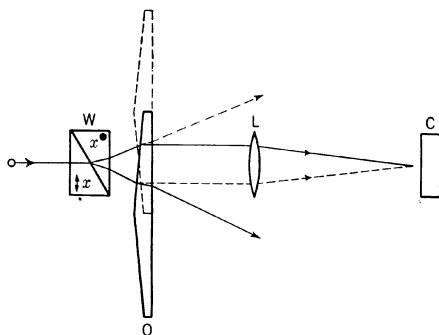


Fig. 79.—Quasi half-shade arrangement of Bruhat and Chatelain.

on rotating the polarizer, first in the absence and then in the presence of the optically active substance. The Wollaston prism, like any other double image prism, is implicitly equivalent to a Jellett-Cornu prism with a half-shade angle of 90° . The lateral displacement of quartz prism *Q*, in conjunction with condensing lens *L*, makes possible receiving alternately one and the other of the two rays, the latter being dotted, in photocell *C*. Interpolation is used, as in the preceding method, to reduce the number of stepwise rotations of the polarizer. With an amplification factor of 10^4 , rotations could be measured in the violet and ultraviolet with an error of 10 – $15'$. In order to remain within the region of linearity between light intensity and photoelectric current, measurements at 4358 \AA . had to be carried out with a light-absorbing screen in the light path.

Flicker Method.—The intensity of light transmitted through the analyzer and, with it, the galvanometer deflection vary twice between zero and maximum if the analyzer is rotated by 360° (page 1523). A continuous

¹⁷¹ G. Bruhat and P. Chatelain, *Rev. optique*, 12, 1 (1933).

rotation of the analyzer therefore causes the galvanometer mirror to vibrate unless the rate of rotation exceeds the inertia of the instrument. The introduction in front of the rotating analyzer of a stationary half-shade device, with a half-shade angle of 90° , *e. g.*, of a Wollaston prism,¹² makes this flicker phenomenon useful in polarimetry (Fig. 80). It is as-

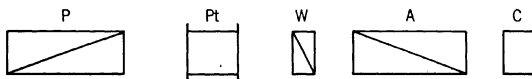


Fig. 80.—Scheme of photoelectric flicker apparatus of Schönrock and Einsporn.

sumed first that the beam incident upon the Wollaston prism, *W*, vibrates under an angle of 45° with respect to the orthogonal optic axes, *i. e.*, that the intensity of the two emerging components is equal. Instead of analyzing the intensity of either component separately, as in the method of Bruhat and Chatelain, both components are sent through the rotating prism, *A*.

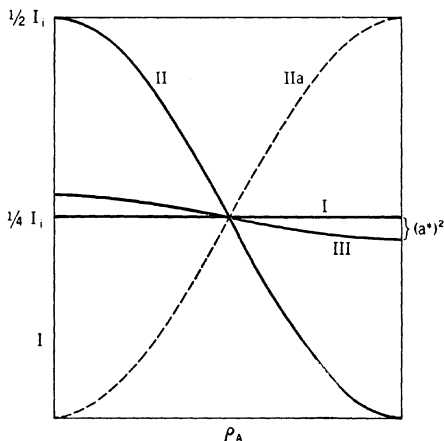


Fig. 81.—Principle of flicker method.

and are received together by the photocell, *C*. According to figure 34a, the combined intensity of the two components is independent of the rotation of the analyzer, ρ_A . The 45° incidence upon the Wollaston prism thus manifests itself by a steady deflection of the galvanometer, which corresponds to $1/4 I_1$ in figure 81, curve I. If an optically active substance

¹² O. Schönrock and E. Einsporn, *Physik. Z.*, **37**, 1 (1936).

is introduced which produces a rotation of 45° , only one component will be transmitted through the Wollaston prism, and the intensity, recorded by the photocell, will vary between $\frac{1}{2} I_1$ and 0 as the result of the analyzer rotation (curve II or IIa of Fig. 81). The polarizer is now rotated until the vibration of the galvanometer deflection ceases. The rotation necessary— 45° in the present example—gives the value of α directly.

The sensitivity of the method is defined by the angle $\Delta\rho_P$, by which the polarizer must be rotated in order to transform an intensity which is constant (curve I of Fig. 81) into one which changes periodically (curve III). The square of the amplitude, a^* , defines the smallest change in light intensity which makes the galvanometer deflection vibrate perceptibly. For a given amplification factor, the sensitivity of the flicker method is obviously identical with that of a quasi half-shade method of the types previously discussed.

Contrary to the preceding quasi half-shade methods, the flicker method does not require a stepwise approach or interpolation of the position of "field equality." A disadvantage, however, is that the analyzer must be kept in continuous rotation at a proper and constant speed in order to prevent an erratic character in the period and amplitude of the vibrations. Schönrock and Einsporn operated with 100 rotations per minute, *i. e.*, a time of 0.3 second was allowed for one period in the vibration of the galvanometer deflections.

In conclusion, it may be added that the flicker method—based on a very old and well-known principle of flicker photometry—is without interest for visual polarimetry because it is equivalent to a half-shade method operated with a half-shade angle of 90° .

Method of Orthogonal Angles.—Almost 70 years ago, Desains^{173, 174} introduced a method in thermoelectric polarimetry which, without alteration, could be used also in photoelectric polarimetry. It will be discussed here because the apparatus needed is very inexpensive; no graduated circles are required. The method operates with a rotation of the analyzer by a fixed angle of 90° . The switch-over from one position to the other can be carried out in rapid succession, by two mechanical stops on the analyzer mounting.

In figure 82, $OT_a = X$ and $OT_b = Y$ are the directions of transmission of the analyzer in its two orthogonal positions. It follows from the figure that

$$\cos^2 \sigma_{0b} = I_{0X}/(I_{0X} + I_{0Y}) \quad (24a)$$

$$\cos^2 \sigma_{1b} = I_{1X}/(I_{1X} + I_{1Y}) \quad (24b)$$

¹⁷³ P. Desains, *Compt. rend.*, **62**, 1277 (1866).

¹⁷⁴ P. Desains, *Compt. rend.*, **84**, 1056 (1877).

Four intensity measurements, or three if $\sigma_{0a} = \sigma_{0b} = 45^\circ$, therefore give the optical rotation $\alpha = \sigma_{1b} - \sigma_{0b}$. Instead of this triple equation, derived by Desains, the more general equation^{164a} may be used:

$$\cos \alpha = (\sqrt{I_{0x}I_{1x}} + \sqrt{I_{0y}I_{1y}})/\sqrt{(I_{0x} + I_{0y})(I_{1x} + I_{1y})} \quad (25)$$

The method of Ingersoll is closely related to that of Desains. It will be discussed in the section on thermoelectric polarimetry (page 1600).

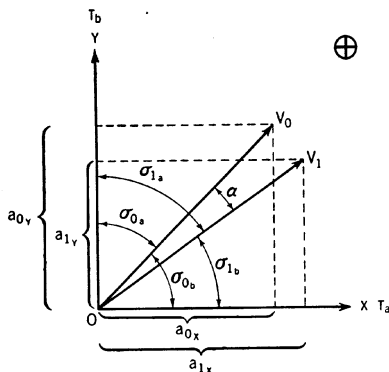


Fig. 82.—Method of "orthogonal angles."

Method of Compensation.—The first photoelectric method introduced into polarimetry¹⁷⁵⁻¹⁷⁷ was based on the principle of compensation (Fig. 83). The polarimeter tube filled with an inactive reference liquid is brought into the light path, and the analyzer or polarizer is rotated until $\sigma_0 = 45^\circ$. The corresponding reading on the graduated scale is ω_0 , and the intensity, I_0 , recorded by the galvanometer is $I_t/2$ in the absence of absorption (curve A). After introduction of the optically active substance, the analyzer or polarizer is rotated until $I_1 = I_0$ (transition from curve A to B). The corresponding reading on the graduated scale is ω_1 . The difference $(\omega_1 - \omega_0) = \alpha$. Instead of rotating the analyzer or polarizer, a quartz compensator could be introduced (page 1553).

A considerable advantage of this over several of the preceding methods and particularly over the method to be discussed on page 1598, is that all measurements are made in the region of maximum sensitivity of the I vs. σ curve regardless of the magnitude of the rotation. A serious disadvantage

¹⁷⁵ H. von Halban and K. Siedentopf, Ger. Pat. 386,537 (1922).

¹⁷⁶ K. Mayrhofer, *Thesis*, Univ. of Würzburg, 1924.

¹⁷⁷ H. von Halban, *Nature*, 119, 86 (1927).

of the method when used in the simplified form described is the falsification of results if the natural rotatory power is accompanied by light absorption, absent in the reference liquid, which reduces I_1 by ΔI . Curve B' is then valid instead of B , *i. e.*, the apparent value of α differs by the decrement $\Delta\rho$ from the true value. Even in the absence of light absorption or light scattering, an error not negligible in precision measurements of small rotations may result if the refractive index of the optically active liquid differs much from that of the reference liquid. For the loss of light by re-

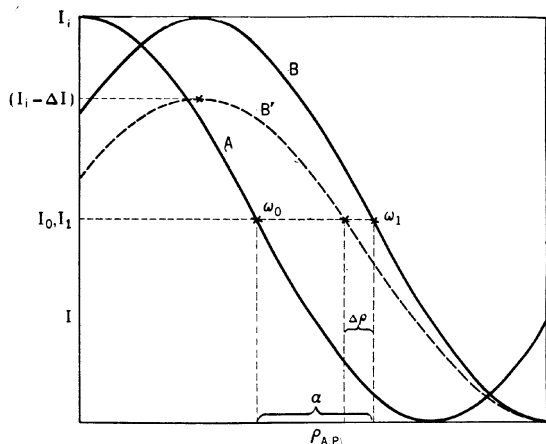


Fig. 83.—Method of compensation.

flection is then different in the two cases. Neither of these disadvantages exists if the method is used for measurements of a Faraday effect because the substance to be studied can be used in zero magnetic field as the reference liquid.

For measurements of the natural rotatory power, von Halban designed an instrument (shown schematically in Fig. 84) which is free from these defects. The light coming from a mercury arc is split into two beams by oblique incidence upon a quartz plate, Q . Under the selected incidence of 45° , the reflected beam carries 10% of the total light flux. One of these beams traverses polarimeter tube Pt_1 , the other Pt_2 . These tubes are of the same length and are filled with an identical liquid. The two photo-cells, C_1 and C_2 , are balanced to zero photoelectric current. After introducing the optically active liquid into both light paths, the balance is re-established by rotating the analyzer, A , or polarizer, P . The reproducible maximum accuracy is given as 0.01° for the strong ultraviolet lines of

the mercury arc. The monochromatic measurements of Mayrhofer¹⁷⁶ have covered the range down to 2536 Å. The method makes use of the photoelectric photometer of von Halban and Siedentopf,¹⁷⁸ and has been altered in matters of detail by Ebert and Kortüm,¹⁶⁷ who discuss the sources of error at length. The apparatus of von Halban gives, in the same set of experiments, both the rotatory power and the light absorption, a welcome possibility which, however, exists also for several other methods discussed above.^{164a}

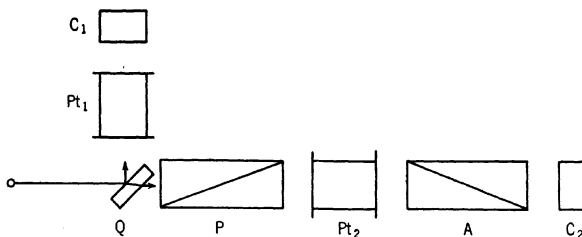


Fig. 84.—Scheme of apparatus of von Halban and Siedentopf.

Scanning Method.—The simplest and, at first thought, most inviting principle of photoelectric polarimetry consists of a scanning of the I vs. σ curve of figure 29 (page 1524). Polarizer and analyzer are in a fixed position defined by $\sigma_0 = 45^\circ$, so that graduated circles are superfluous, as in the method described on page 1595. If light absorption, light scattering, and reflectivity of the reference substance and of the substance to be investigated are the same, the rotatory power is obtained immediately from the intensities I_0 and I_1 recorded by the photocell. For, if $\sigma_0 = 45^\circ$:

$$\pm \sin 2\alpha = (I_1 - I_0)/I_0 \quad (26)$$

as already derived by Desains,¹⁷⁹ who introduced the scanning method in thermoelectric polarimetry. Todesco^{180, 181} introduced the scanning method in photoelectric polarimetry of the Faraday effect.

The method, in the simple form outlined, is most appropriate for the study of a weak Faraday effect where $\sigma_1 \approx \sigma_0 = 45^\circ$. In measurements of a well-pronounced natural rotatory power, one must contend with the two inherent disadvantages of the method, one due to the interference of absorption or light scattering and the other, to the fact that $\sigma_1 \neq 45^\circ$. The

¹⁷⁶ H. von Halban and K. Siedentopf, *Z. physik. Chem.*, **100**, 208 (1922).

¹⁷⁹ de La Provostaye and P. Desains, *Ann. chim. phys.*, **27**, 232 (1849); **30**, 267 (1850).

¹⁸⁰ G. Todesco, *Nuovo cimento*, **5**, 376 (1928).

¹⁸¹ E. Perucca, *Atti reale accad. Lincei, Classe sci fis mat. nat.*, **7**, 733 (1928).

former, more important, disadvantage has been eliminated by the design of an apparatus shown schematically in figure 85.^{182, 183} In a first pair of experiments, (a), in the absence and the presence of the optically active substance, the analyzer, A , is set between polarizer P and polarimeter tube Pt . From the data, I_{0a} and I_{1a} , the absorption effect can be calculated. In a second pair of experiments, (b), the analyzer is switched to its normal position. From the data, I_{1a} and I_{1b} , the correct optical rotation follows. Instead of permanently setting the polarizer and analyzer for an angle $\sigma = 45^\circ$, as proposed above, the authors preferred to have the nicols permanently crossed and to insert a quartz plate, Q , cut perpendicular to the optic axis and producing a rotation of, and consequently σ_0 of, 34.65° for the D

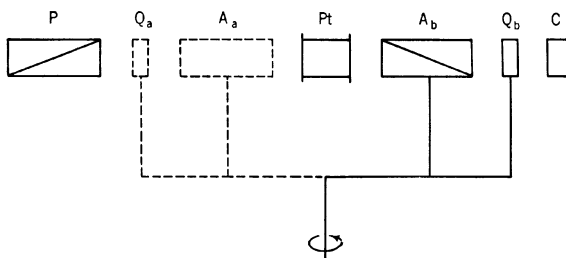


Fig. 85.—Scheme of apparatus of Landt and Hirschmüller.

line. Very small rotations can therefore not be measured with the same degree of accuracy as is possible if $\sigma = 45^\circ$. On the other hand, the accuracy is higher than in the alternate case if the rotations exceed $\sim 10^\circ$. On the assumption—which requires verification—that the switch-over of the analyzer from position (a) to position (b) can be achieved without a detectable change in the azimuth, the only weakness of this modified scanning method consists of a variation in the accuracy of the measurements with the magnitude of the rotation. This problem and its possible partial solutions are identical with those existing for the method of the orthogonal angles (page 1595).^{164a} The instrument discussed is designed for technical measurements and is fitted with a Herotar polarizer and analyzer,^{184, 185} products similar to the Polaroid.

¹⁸² E. Landt and H. Hirschmüller, *Deut. Zuckerind.*, **62**, 647 (1937).

¹⁸³ E. Landt, H. Hirschmüller, and W. Bechstein, German Pat., assigned to Schmidt and Haensch, No. 691,441, April 30, 1940.

¹⁸⁴ F. Bernauer, *Fortschr. Mineral. Krist. Petrog.*, **19**, 22 (1935).

¹⁸⁵ M. Haase, *Zeiss Nachr.*, **2**, 55 (1936).

C. PHOTOELECTRIC SPECTROPOLARIMETRY

Several of the methods described above have been used for determining the dispersion of the rotatory power by means of a series of successive measurements at various wave lengths. Since it is impossible to imitate those visual or photographic spectropolarimetric methods in which a spectral record is examined, it is necessary to base any photoelectric dispersion measurements on successive measurements. This necessity does not exclude, however, the introduction into photoelectric spectropolarimetry of typically spectropolarimetric principles. No literature appears to exist in this respect. The methods which might be used will follow from the discussion of thermoelectric spectropolarimetry (page 1602), a branch of spectropolarimetry which is well developed. The possibilities and restrictions in photoelectric spectropolarimetry are the same as in thermoelectric spectropolarimetry, with a few exceptions stated below.

10. Thermoelectric and Bolometric Polarimetry and Spectropolarimetry

Thermoelectric and bolometric polarimetry and spectropolarimetry date back almost as early as visual polarimetry and spectropolarimetry. The first observations in the infrared were made in 1836¹⁸⁶ and 1846¹⁸⁷ on the natural rotatory power and on the Faraday effect, respectively. As early as 1849, Desains carried out quantitative measurements of either effect in the infrared by means of methods discussed on pages 1595-1598.

A. THERMOELECTRIC AND BOLOMETRIC POLARIMETRY

Thermoelectric and bolometric polarimetry are closely related to photoelectric polarimetry. In either case, measurements of a rotation are based on the recording of an electric current or of an electric potential as produced or changed by illumination or irradiation. All methods of photoelectric polarimetry are therefore directly applicable to thermoelectric and bolometric polarimetry. In fact, several methods developed in thermoelectric polarimetry and applicable to photoelectric polarimetry have been discussed in the section on photoelectric polarimetry because the latter has a more general interest. It is therefore sufficient to discuss a few important details here.

Ingersoll¹⁸⁸ adapted the method of orthogonal angles to measurements with a differential bolometer. The use of a differential bolometer or differential thermocouple is, in fact, less objectionable than the use of differential

¹⁸⁶ J. B. Biot and Melloni, *Compt. rend.*, 2, 194 (1836).

¹⁸⁷ Wartmann, *Compt. rend.*, 22, 556, 745 (1846).

¹⁸⁸ L. R. Ingersoll, *Phys. Rev.*, 23, 489 (1906); *Phil. Mag.*, 11, 41 (1906).

photocells. A Wollaston prism is employed as the analyzer, as in the method of Bruhat and Chatelain (page 1593). Each of the two emerging rays is incident upon one of the two bolometers, and instead of two intensities, I_X and I_Y , the intensity difference ($I_X - I_Y$) is measured (Fig. 82). If $\sigma_0 = \sigma_0 = 45^\circ$, the intensity difference is zero in the absence of the optically active substance. The galvanometer deflection obtained after introduction of the optically active substance is proportional to $I_{1X} - I_{1Y}$ (see Fig. 82). Since the numerical values of I_{1X} and I_{1Y} must be known for the calculation of α , a second differential measurement is necessary after rotating the polarizer by 90° . The number of measurements, three, is therefore not reduced compared with the original method of the orthogonal angles if the latter is operated with $\sigma_0 = \sigma_0 = 45^\circ$.

Of particular technical interest is the infrared polarimeter designed by Meyer.¹⁸⁹ It was used for investigating the Faraday effect in the far-

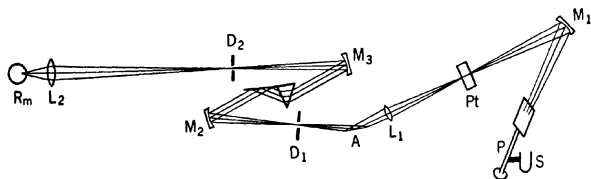


Fig. 86.—Meyer's apparatus for spectropolarimetry in the far-infrared.

infrared, but it could also be used for investigating the natural rotatory power. A scheme of the apparatus is given in figure 86.

S represents a Nernst lamp and P a polarizing mirror. Other symbols are: M_1 , M_2 , M_3 —concave mirrors; A —analyzing mirror; D_1 , D_2 —diaphragms; L_1 , L_2 —sodium chloride lenses; R_m —radiomicrometer. The instrument parts between and including D_1 and D_2 represent a Wadsworth spectroscope (see page 1602).

Both the polarizer and the analyzer are reflecting mirrors. The rotation is measured by the extinction method: The polarizing mirror is rotated until the galvanometer deflection reaches a value of zero. In order to maintain the relative position of the source of radiation (Nernst lamp) with respect to the polarizing mirror, lamp and mirror are mounted together rigidly and rotated around the direction of the reflected beam as the axis. Meyer extended his measurements up to a wave length of $88,500 \text{ \AA.}$, which has not been exceeded in any of the later measurements on either the Faraday effect or the natural rotatory power.

¹⁸⁹ U. Meyer, *Ann. Physik*, 30, 607 (1909).

B. THERMOELECTRIC AND BOLOMETRIC SPECTROPOLARIMETRY

Methods employing a single thermopile or a single bolometer require successive measurements, as do photoelectric methods. Such methods were used particularly by Meyer¹⁸⁹ and Ingersoll.¹⁸⁸ In both instances, the polarimeter was followed by an infrared monochromator consisting of a prism-mirror combination of constant deviation of the Wadsworth¹⁹⁰ type. This combination is preferable to the Pellin-Brocca prism usually found in monochromators and spectrographs, if the radiation is partly absorbed by the substance of which the prism is made.

Introduction of the method of Fizeau and Foucault into thermoelectric spectropolarimetry is due to Carvallo,^{191, 192} who used a double purpose prism of the Joubin type¹⁹³ to serve both as an analyzer and as a dispersing prism. The polarizer was rotated until a fringe or its complement, a spectral range of maximum transmission, passed the thermopile. This method is equivalent to the original method of Fizeau and Foucault (page 1562), which is useful if the rotations are very large, *i. e.*, if the fringes are narrow (page 1568). Lowry and Coode-Adams¹⁹⁴ improved the limited accuracy of results obtained by this method by making two series of extinction (fringe) and intensity maxima determinations. In the second series, the polarizer was rotated by 90° so that the maxima of the first series appeared as fringes and vice versa. By taking the mean values the centers of the maxima and minima, respectively, are obtained somewhat more accurately. Instead of rotating the polarizer and keeping the wave length constant, in a given measurement, an arbitrary polarizer position may be chosen, *e. g.*, the one corresponding to crossed nicols, and the wave length may be varied instead (see page 1565). An improved version of this alternate method has been used by Lowry and Snow,¹⁹⁵ who measured the infrared rotation of quartz at regular spectral intervals of 100 Å. between 18,000 and 27,900 Å.

By employing differential thermocouples or bolometers, additional methods are possible in thermoelectric spectropolarimetry, such as the Lippich modification of the method of Fizeau and Foucault (page 1564). Dongier¹⁹⁶ used such a differential method. Each of the two juxtaposed spectra (see Fig. 61, page 1564) is focused upon one of the two thermoele-

¹⁸⁹ F. L. O. Wadsworth, *Phil. Mag.*, **38**, 337 (1894); *Astrophys. J.*, **1**, 232 (1895).

¹⁹¹ E. Carvallo, *Ann. chim. phys.*, **26**, 113 (1892).

¹⁹² See also G. Moreau, *Ann. chim. phys.*, **30**, 433 (1893).

¹⁹³ P. Joubin, *Ann. chim. phys.*, **16**, 78 (1889). See this chapter, pp. 1519 and 1580.

¹⁹⁴ T. N. Lowry and W. R. C. Coode-Adams, *Proc. Roy. Soc. London*, **A226**, 391 (1927).

¹⁹⁵ T. M. Lowry and C. Snow, *Proc. Roy. Soc. London*, **A127**, 271 (1930).

¹⁹⁶ R. Dongier, *Ann. chim. phys.*, **14**, 331 (1898); *J. phys.*, **7**, 637 (1898); *Bull. soc. franc. phys.*, **105**, 1 (1898).

ments. The wave-length drum of the monochromator is rotated until the galvanometer deflection reaches zero. The wave length fulfilling this condition is λ_{Eq} . Dongier used a Wollaston prism as the analyzer so that the mutual displacement, 2ϵ , of the fringes in the two juxtaposed spectra amounted to 90° . This angle is evidently the optimum condition for accuracy, just as in photoelectric polarimetry (page 1592).

As stated on page 1600, the methods in thermoelectric and bolometric spectropolarimetry are directly applicable to photoelectric spectropolarimetry with certain exceptions. These exceptions are methods, as that of Dongier, involving differential receiving instruments, which are not advisable in photoelectric polarimetry. By using the flicker method (page 1593), such methods could be adapted, however, to photoelectric spectropolarimetry.

11. Polarimetry and Spectropolarimetry by Means of Fluorescent and Phosphorescent Screens

A. APPLICATION OF FLUORESCENCE

An ultraviolet line spectrum made visible by means of a fluorescent screen will stand out on a dark, nonfluorescent background. Conversely, a Fizeau-Foucault fringe becomes a dark band on a fluorescent background if a continuous ultraviolet spectrum is used. Fluorescent screens therefore make possible the use of visual methods of polarimetry and spectropolarimetry for investigating the ultraviolet. Soret and Sarasin¹⁹⁷ employed fluorescent screens of uranium glass or impregnated with esculin in the first experiments ever carried out on natural rotatory power in the ultraviolet, and studied it by means of Fizeau-Foucault fringes using the line spectrum of a spark (see page 1577). This method was again applied by Duclaux and Jeantet,¹⁹⁸ but accurate measurements are not possible with it because of the somewhat diffuse character of the spectral lines obtained on a fluorescent screen and because of the enhancement in the diffuse character of the Fizeau-Foucault fringes made visible by fluorescence. If the rotations are very large, *i. e.*, if the number of fringes is high, this inaccuracy is of minor importance. Fluorescence may be used not only for ultraviolet spectropolarimetry, but also for ultraviolet polarimetry. A fluorescent screen is set into the eyepiece of the telescope used for observing the optical field (Fig. 53, page 1553).¹⁹⁷ If the polarimeter operates on the principle of the half-shade, the angle of the latter must be large in order to obtain a perceptible fluorescence in the position of field equality. This makes accurate measurements impossible.

Fluorescent screens can therefore not be used for precision polarimetry and spectropolarimetry; but they are of high practical value for exploratory experiments

¹⁹⁷ J. L. Soret and E. Sarasin, *Compt. rend.*, 95, 636 (1882); 84, 1362 (1877); 83, 818 (1876); 81, 610 (1875); *Arch. sci. phys. nat.*, 8, 5, 97, 201 (1882).

¹⁹⁸ J. Duclaux and P. Jeantet, *J. phys. radium*, 2, 156 (1921).

in the ultraviolet and for the adjustment of ultraviolet polarimeters and spectropolarimeters, as demonstrated particularly by Bruhat.^{199, 200}

B. APPLICATION OF PHOSPHORESCENCE

While fluorescence persists only as long as the exciting light is absorbed, phosphorescence may persist for a considerable time after the irradiation has ceased. Inasmuch as infrared radiation extinguishes phosphorescence, a phosphorescent screen can be used in infrared polarimetry and spectropolarimetry. The phosphorescence is produced by ultraviolet radiation; the phosphorescent screen is then installed in an infrared polarimeter or spectropolarimeter; and, after the exposure, a negative of the actual infrared pattern will be visible on the phosphorescent screen. Fizeau-Foucault fringes, for example, are indicated by phosphorescent patches in a dark field; spectral lines stand out as dark lines on a phosphorescent background. Hussell,²⁰¹ whose method of infrared spectropolarimetry was original also in other respects (see page 1583), used phosphorescent screens in infrared spectropolarimetry. A permanent record of the pattern may be obtained by bringing the phosphorescent screen into contact with a photographic plate. All remarks made above about the value of fluorescent screens for polarimetry and spectropolarimetry apply also to phosphorescent screens.²⁰²

VI. STANDARD CONDITIONS FOR DETERMINING OR EXPRESSING ROTATORY POWER

The optical rotation exhibited by an optically active solid or liquid of a given dissymmetric structure depends on the layer thickness, the wave length, and the temperature. The optical rotation exhibited by a solution of an optically active substance furthermore depends on the concentration of the optically active substance and the nature of the optically inactive medium in which the substance is dissolved. In certain cases, optical activity changes with time. The time elapsed since the dissolution of the optically active substance or since a change in physical conditions (*e. g.*, temperature) or chemical conditions (*e. g.*, pH) may thus play an important role. Racemization and mutarotation are the best-known examples of variable rotatory power due to the latter changes. In order to make the observation of a rotation significant, it is therefore indispensable to define the experimental conditions. In addition, and as far as possible, the experimental rotations should be reduced to standard values.

¹⁹⁹ G. Bruhat and M. Pauthenier, *Rev. optique*, **6**, 163 (1927).

²⁰⁰ For information on the preparation, properties, and use of fluorescent screens, the reader is referred to: P. Pringsheim and M. Vogel, *Luminescence of Liquids and Solids and Its Principal Practical Applications*, Interscience, New York, 1946.

²⁰¹ A. Hussell, *Ann. Physik Chem.*, **43**, 498 (1891).

²⁰² For further information on phosphorescence and on phosphorescent screens, see P. Pringsheim, *Fluoreszenz und Phosphoreszenz*, Springer, Berlin, 1925.

The standard layer thickness is 10 cm. Since the rotation is proportional to the layer thickness (page 1496), any convenient layer may be used for the experiments and the standard value calculated.

For a long time, the sodium doublet (D line, 5890, 5896 Å.) was used as the standard wave length in the visible, and is still being used by many authors. In accordance with the suggestion by Bates²⁰³ for changing the standard, the green mercury line (5461 Å.) has been favored increasingly. The latter line is in fact preferable because of the doublet character of the former. It must be emphasized, however, that indicating the rotation for one wave length has only a limited significance, particularly if two or more substances are to be compared. Whenever possible, the character of the dispersion of the rotatory power should be determined within at least a spectral range of 1000 Å. Many substances which do not differ characteristically in their rotation at a certain wave length show definite differences in the dispersion of the rotation.

In a spectral region of normal dispersion, Drude's theoretical equation is valid in a first approximation:

$$[\alpha] = A/(\lambda^2 - \lambda_c^2) \quad (27)$$

where A is a constant, λ_c is the wave length characterizing the nearest optically active absorption band, and $[\alpha]$ is defined by equation (29). It thus is possible to determine λ_c , which generally is in the ultraviolet, if sufficient data on the dispersion are collected. For merely practical purposes, Biot's empirical rule:

$$[\alpha] = A'/\lambda^2 \quad (28)$$

can often be used with a fair degree of approximation if the dispersion is normal in the region considered and if $\lambda_c \ll \lambda$.

While dispersion measurements are most advisable in studies on pure substances, they become indispensable in studies of mixtures, excepting very simple cases such as mixtures of sugars. Mixtures of optically active substances in a solution can often be recognized only by their dispersion, particularly by means of the Darms diagram.²⁰⁴ In order to take advantage of the analytical value of dispersion measurements, it is not always necessary to compile the whole dispersion curve. Measurements at two sufficiently distant wave lengths are often sufficient for the purpose. The following wave lengths are favored combinations for obtaining the "dispersion ratio," $\alpha_{\lambda_2}/\alpha_{\lambda_1}$: 4360 and 5461 (both mercury lines); 4360 (mercury line) and 5893 (D line); 5085 and 6438 Å. (both cadmium lines).

Rotations are usually determined at 20° C. (68° F.). Since the sensitivity of the rotatory power toward temperature changes varies from sub-

²⁰³ F. J. Bates, *Natl. Bur. Standards U. S. Bull.*, 2, 239 (1906).

²⁰⁴ E. Darms, *Ann. chim. phys.*, 22, 247 (1911).

stance to substance, a reduction of values obtained at another temperature to 20° C. is not possible with accuracy unless the temperature function is known by experiments or from the literature.

The concentration does not enter into consideration if the optically active substance is not a solution but a liquid. However, the same layer generally contains less substance at a higher temperature than at a lower temperature, due to dilation; and as a general practice, introduced by Biot (1835), this quasi concentration effect is excluded by considering unit density. The *specific* rotation is therefore defined by:

$$[\alpha] = \alpha/bd \quad (29)$$

where b is the experimental layer thickness, in decimeters, and d is the density. If the rotatory power of different substances is to be compared, it is generally more significant to calculate the molecular rotation, expressed as the *molecular* rotatory power:

$$[M] = (M/100)[\alpha] \quad (30)$$

where M is the molecular weight.

The values of $[\alpha]$ are ten times larger than the unused unit of the c.g.s. system; the values of $[M]$ are ten times smaller than the c.g.s. unit. The departure from the c.g.s. system is, in both cases, caused by the desire for values of a convenient order of magnitude.

It might be advisable to use equations (29) and (30) also for optically active solids in order to make a significant comparison possible between the rotatory powers of the same substance in the solid and liquid state.

In the case of solutions, the specific rotation is defined, in agreement with the preceding definition, as the rotation exhibited by one gram of optically active substance in one cubic centimeter of solution. If c is the concentration, in terms of grams of optically active substance per 100 cc. of solution:

$$[\alpha] = 100\alpha/bc \quad (31)$$

If the solution is known in terms of grams of optically active substance per 100 g. of solution (p):

$$[\alpha] = 100\alpha/bpd \quad (32)$$

where d is the density of the solution for the temperature considered. For $pd = c$. The temperature and wave length are indicated by a superscript and a subscript, respectively. Thus, a specific rotation valid for 5890-5896 Å. and 20° C. is written $[\alpha]_{20}^{5890}$.

In contradistinction to the specific or molecular rotation of solids or liquids, the specific or molecular rotation of solutions is not a true constant. Biot's rule on the proportionality between the rotation and concentration holds with a satisfactory approximation only in very dilute solutions. A single value on the specific or molecular rotation of solutions, calculated with equation (31) or (32) is therefore significant only if the concentration is stated. Particularly, one procedure has been used by a number of authors in order to arrive at data of more general significance. It consists of an extrapolation of the rotation to a 100% concentration. This, however, is safe only if a sufficient number of data are available at high concentrations. It is more significant to use the reverse procedure, *i. e.*, an extrapolation to infinite dilution. If the rotatory power is to be used for the determination of concentrations, *e. g.*, in saccharimetry, an empirical function satisfying the experimental $[\alpha]$ vs. c curve must be established. Since the curves representing the variation of the rotatory power with the concentration are generally free from minima and maxima, an equation of three terms:

$$[\alpha] = A + Bc + Cc^2 \quad \text{or} \quad [\alpha] = A' + B'q + C'q^2 \quad (33)$$

where q is the concentration of the optically inactive medium, in per cent of the total weight, can be made to fit the experimental curve in the majority of cases by choosing the appropriate constants A (A'), B (B'), and C (C'). Among these, constants A and A' should obviously represent the specific rotation at infinite dilution and of the pure optically active substance, respectively. Unfortunately, this is not always strictly fulfilled. In some cases, a nearly linear relationship exists between $[\alpha]$ and the concentration. The third term of equation (33) is then superfluous. The examples of nicotine in ethanol in table X and of sucrose in water in table XI may serve to show the practical value of equations of the type of (33).

TABLE X
VARIATION OF SPECIFIC ROTATION, $[\alpha]$, WITH CONCENTRATION OF NICOTINE
IN ETHANOL*

Ethanol concentration, q	$-\alpha_D^{20}$	
	Experimental	Calculated
0.00	161.55°	...
9.91	158.65°	158.63°
25.07	154.92°	155.26°
40.07	151.78°	151.92°
69.97	145.42°	145.27°
85.04	141.60°	141.92°

* Data from H. Landolt, *Ann.*, 189, 241 (1877), the empirical equation being: $-\alpha_D^{20} = 160.83 - 0.2224 q$.

TABLE XI
 VARIATION OF SPECIFIC ROTATION, $[\alpha]$, WITH CONCENTRATION OF SUCROSE IN WATER*

Water concentration, g	$[\alpha]_D^{20}$	
	Experimental	Calculated
35.022	65.620°	65.619°
60.022	66.272°	66.242°
83.007	66.448°	66.506°
95.002	66.609°	66.526°

* Data from H. Landolt, *Das optische Drehungsvermögen organischer Substanzen*, Vieweg, Braunschweig, 1898. Empirical equation: $[\alpha]_D^{20} = 64.156 + 0.051596 q - 0.0002805 q^2$.

An intrinsic peculiarity of empirical equations involving several constants is that different equations with different constants may satisfy a wide range of the same curve. This is illustrated by the following four equations proposed for aqueous solutions of sucrose in addition to that used in table XI. The equation of Landolt (1898) in its revised form, given below, is accepted as the standard equation in saccharimetry.

$$[\alpha]_D^{20} = 66.462 + 0.0087 c - 0.000235 c^2 \quad \begin{array}{l} \text{(Landolt)} \\ \text{(valid for } c \text{ values between 0 and 65)} \end{array} \quad \begin{array}{l} \text{(revised)} \end{array}$$

$$[\alpha]_D^{20} = 66.438 + 0.010312 p - 0.0003545 p^2 \quad \text{(Nassini and Villavecchia)}$$

$$[\alpha]_D^{20} = 63.904 + 0.064686 q - 0.0003986 q^2 \quad \begin{array}{l} \text{(Tollens)} \\ \text{(valid for } q \text{ values between 31 and 96)} \end{array}$$

$$[\alpha]_D^{20} = 64.190 + 0.055212 q - 0.0003134 q^2 \quad \text{(Thomsen)}$$

There is, of course, no standard medium, since the medium to be used depends on the solubility of the optically active substance. Water is most often used, besides alcohol, benzene, and other organic solvents. There are several rules on the variation of the specific rotation with the medium, particularly with regard to the polarity of the solvent; but no satisfactory quantitative relationship has yet been found. A specific rotation, $[\alpha]_D^{17}$, is therefore significant only if the medium and the concentration are stated, unless the specific rotation is extrapolated for 0 or 100% concentration. The latter is possible with readily soluble substances, but the latter method of extrapolation should even then be used with precaution. A few values for nicotine may be given as an example of a favorable case. Its specific optical rotation at 20° C. (D line) is, at 100% concentration, -161.55° , -160.83° , and -161.29° , if determined in the pure liquid, by extrapolation from a solution in ethanol, and from a solution in water, respectively.

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Determination of DIPOLE MOMENTS

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I. USE OF DIPOLE MOMENT

This chapter is not a monograph on the subject of dipole moments nor even a complete account of the details of all the methods which might conceivably be employed for the measurement of dipole moments. It aims to outline briefly the theory of the molecular dipole, to illustrate the manner in which dipole moment determinations may be applied to structural problems, to summarize and evaluate the possible but less desirable methods of measurement, and to describe in detail examples of methods which, at the time of writing, seem to be the most convenient and accurate.

The dipole moments of molecules have been employed principally in the determination of the locations of the atoms in molecules and of the effects of resonance through the contributions of polar structures to the molecular structure. A dipole consists of two equal electric charges, very close together and opposite in sign. Its magnitude is measured by its moment, which is the product of either charge by the distance between the two charges. As the moment acts in the direction of the line joining the two charges, that is, the axis of the dipole, it is a vector quantity. The hydrogen chloride molecule is a simple example of a molecular dipole.

which, in this case, lies in the H—Cl bond with its positive end toward the H and its negative toward the Cl. This further illustrates the fact that a chemical bond between atoms of different elements normally has a dipole moment acting in the direction of the bond. The moment of a molecule is the vector sum of all the individual moments in its structure; but, in many molecules, one or two dipoles are so much larger than the others that the moments of the latter may, as a satisfactory approximation, be disregarded or treated as part of those of the large dipoles.

The order of magnitude of molecular dipole moments is given by the product of the electronic charge, 4.80×10^{-10} electrostatic units, times an interatomic distance of the order of 10^{-8} cm. If, for example, the hydrogen chloride molecule is treated as consisting of a proton and a chloride ion separated by the internuclear distance 1.28×10^{-8} cm. (calculated from infrared spectra), the estimated dipole moment is $(4.80 \times 10^{-10}) \times (1.28 \times 10^{-8}) = 6.14 \times 10^{-18}$. This value calculated for an ionic hydrogen chloride structure is much larger than the experimental value, 1.04×10^{-18} , obtained from the measurement of the dielectric constant of the vapor over a range of temperature. It has been shown¹ that this discrepancy can be explained in terms of the distortion of the electronic arrangement of the chloride ion by the attractive force exerted by the proton, which shortens the resultant dipole. A different and quite useful approach to the problem, in terms of resonance, is to treat the structure of the hydrogen chloride molecule as a linear combination of an undistorted ionic structure H^+Cl^- of moment 6.14×10^{-18} as just calculated and a purely covalent structure H—Cl of zero moment. The hydrogen-chlorine bond is then described as possessing an amount of ionic character given by the ratio of the observed moment, 1.04×10^{-18} , to the calculated value, 6.14×10^{-18} , or $1.04 \times 10^{-18}/6.14 \times 10^{-18} = 0.17$. This method can generally be used for calculating the amount of ionic character in a bond.²

The determination of the geometrical structure of a molecule from its moment commonly consists of calculating the resultant moment for the different possible molecular models and adopting as correct that model which gives a moment value reasonably close to the observed. If the geometrical structure is known, any considerable difference between the calculated and the observed values may be used as a means of investigating the effects of resonance or of steric repulsion within the molecule. In view of the large difference commonly occurring among the moments of isomers, the determination of the dielectric constant may sometimes be used as a means of analyzing mixtures of two isomers.

¹ C. P. Smyth, *Phil. Mag.*, **47**, 530 (1924).

² L. Pauling, *The Nature of the Chemical Bond*. Cornell Univ. Press, Ithaca, 1940, p. 46.

The simplest possible use of the dipole moment in the determination of structure is illustrated by the fact that the determination of a considerable dipole moment for the water molecule showed¹ the impossibility of the symmetrical linear structure previously attributed to it and indicated the probability of directed valence for the oxygen atom. The good-sized dipole moment of *cis*-dichloroethylene as contrasted to the zero moment of the *trans* compound² confirmed the plane structures previously attributed to these molecules. The treatment of the moment, m , of the chlorobenzene molecule as being that of a single dipole in the C—Cl bond made possible the calculation of the angle θ between the directions of the C—Cl bonds in the three dichlorobenzenes from the molecular moments,^{3,4} which should equal $2m \cos (\theta/2)$. This determination of θ indicated the planar structure of the benzene ring given by the Kekulé formula. Comparison⁵ of the moment of chlorobenzene with those of the alkyl chlorides shows that the former is lowered by contributions from structures in which a positively charged chlorine is linked to the ring by a double bond. The extent of the lowering can then be used to calculate⁶ the contributions of the different polar structures, the moments of which can be roughly estimated from the molecular dimensions.

Although values have been calculated for the moments associated with bonds in polyatomic molecules, a bond moment is merely a measure of the electrical asymmetry of a certain section of a molecule and is affected by the environment of the section. The use of a moment found for a bond in one environment for the same bond in a different environment may lead to incorrect conclusions. Thus, two or more dipoles attached to the same atom or to immediately adjacent atoms tend to alter one another's moments by mutual inductive effects. For use in the calculation of molecular dipole moments, a more practically applicable quantity is the group moment, values of which are listed in table I. Each moment value given is that which is found when a group given in the first column is attached to one of the groups listed across the top of the table. For example, the moment given for C_6H_5-Cl is that of chlorobenzene and includes the small moment of the H—C bond and the resonance effects previously mentioned. The moment of *p*-chloronitrobenzene should, in the absence of mutual effects of the two substituents, be $4.21 - 1.72 = 2.49$. The observed⁷ value, 2.78, is higher by a small amount, which can be regarded as a measure of the increase in resonance resulting from the mutual effects of

¹ J. Errera, *Physik. Z.*, **27**, 764 (1926); **29**, 689 (1928).

² C. P. Smyth and S. O. Morgan, *J. Am. Chem. Soc.*, **49**, 1030 (1927).

³ L. E. Sutton, *Proc. Roy. Soc. London*, **A133**, 668 (1931).

⁴ C. P. Smyth, *J. Am. Chem. Soc.*, **63**, 57 (1941).

⁵ L. G. Groves and S. Sugden, *J. Chem. Soc.*, 1937, 1782.

TABLE I
 GROUP MOMENTS ($\times 10^{18}$)

Group	C ₆ H ₅ —		CH ₂ —		C ₂ H ₅ —		Angle
	Gas	Soln.	Gas	Soln.	Gas	Soln.	
—CH ₃	0.36	0.4	0	..	0	..	180°
—OCH ₃	1.35	1.25	1.30	55°
—SCH ₃	..	1.27	..	1.40	(52°)
—NH ₂	1.48	1.53	1.23	..	1.2	1.38	...
—I	1.6	1.30	1.64	1.5	1.87	1.8	0°
—Br	1.75	1.52	1.80	1.8	2.01	1.9	0°
—Cl	1.72	1.55	1.87	1.7	2.05	1.8	0°
—F	1.57	1.43	1.81	..	1.92	..	0°
—OH	1.4	1.6	1.69	1.66	1.69	1.7	62°
—COOH	..	1.7	1.73	1.6	1.73	1.7	74°
—COOCH ₃	..	1.9	1.67	1.75	1.76	1.9	70°
—CHO	(3.1)	2.8	2.72	2.5	2.73	2.5	58°
—COCH ₃	3.00	2.9	2.84	2.74	2.78	..	59°
—CN	4.39	4.0	3.94	3.4	4.00	3.57	0°
—NO ₂	4.21	3.98	3.50	3.1	3.68	3.3	0°

the two substituents.⁸ The moment of *o*-dichlorobenzene should be $2 \times 1.72 \cos 30^\circ = 2.98$. The observed⁹ value, 2.51, is lower by an amount which is primarily a measure of the mutual inductive effects of the two C—Cl dipoles. The observed values for *m*-dichlorobenzene and *m*-chloronitrobenzene, in which mutual effects of resonance and of dipole-dipole induction can play little part, are in excellent agreement with the calculated values.

Two values are listed for each moment, one (Gas), the true value measured in the vapor state, and the other (Soln.), the value obtained from solution measurements, usually affected by mutual inductive effects between solvent and solute molecules. The solution values are the ones to be used for the calculation of moments measured in solution as similar errors are then contained in both the observed and the calculated values. The moment of an ethyl compound, which is frequently higher than that of the corresponding methyl compound, can be used for the group attached to any aliphatic residue, as additional carbon atoms normally affect the moment little. The solution values are frequently given to only one decimal place because the experimental determinations often vary by as much as 0.1 among different investigators and different nonpolar solvents, benzene being the most generally used solvent. The order of magnitude of the moments is 10^{-18} , the product of electronic charge, 10^{-10} e. s. u., by intramolecular distance, 10^{-8} cm. The factor 10^{-18} is commonly omitted in speaking of the values, which thus become expressed

⁸ C. P. Smyth and G. L. Lewis, *J. Am. Chem. Soc.*, **62**, 721 (1940).

⁹ E. C. Hurd and C. P. Smyth, *J. Am. Chem. Soc.*, **64**, 2212 (1942).

in debye units (1 debye = 10^{-18} e. s. u. \times cm.). As most of the group moments are the vector sums of the moments of two or more dipoles, the angle which the + to - direction of this resultant moment makes with the direction of the bond of the carbon atom to the group in question is listed in the last column. Since rotation of the group around the axis of this bond will change and make variable the direction of the group moment when the angle is other than 0° or 180° , groups whose moments lie in the direction of the bond of the carbon atom to the group should be used as far as possible for structural investigations. Rotation around single bonds, such as C—C, separating two groups in a molecule also introduces variability in the direction of the group moment. When complete freedom of rotation is possible about the bonds intervening between two groups, the resultant of the group moments can be calculated by averaging over all possible positions around the bonds.^{10, 11} Serious deviations from the calculated results are then taken as evidence of restriction of rotation and may be used as a means of calculating the extent of the restriction.¹²

The simplest way to calculate the moment of a molecule containing several group moments in fixed positions is to establish a convenient set of reference axes in the molecule, calculate the component of each group moment along each axis, and obtain the resultant moment as the square root of the sum of the squares of the component sums, m_x, m_y, m_z , along each axis, that is, $\mu = (m_x^2 + m_y^2 + m_z^2)^{1/2}$. When only two group moments, m_1 and m_2 , are involved, this method gives the usual expression for the resolution of two vectors, m_1 and m_2 , with an angle θ between them, that is

$$\mu = (m_1^2 + m_2^2 + 2m_1m_2 \cos \theta)^{1/2}$$

The method of calculation may be illustrated by the case of the moment of the *m*-chloronitrobenzene molecule in the vapor state. Setting $m_1 = 1.72$, the moment of chlorobenzene, and $m_2 = 4.21$, the moment of nitrobenzene from the second column of table I, $\theta = 120^\circ$, and letting the x and y axes lie in the plane of the benzene ring with the C—Cl line in the y axis:

$$m_x = m_2 \sin \theta, m_y = m_1 + m_2 \cos \theta, \text{ and } m_z = 0$$

and:

$$\begin{aligned} \mu &= (m_2^2 \sin^2 \theta + m_1^2 + 2m_1m_2 \cos \theta + m_2^2 \cos^2 \theta)^{1/2} \\ &= (m_1^2 + m_2^2 + 2m_1m_2 \cos \theta)^{1/2} \\ &= 3.67 \end{aligned}$$

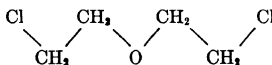
¹⁰ H. Eyring, *Phys. Rev.*, **39**, 746 (1932).

¹¹ C. P. Smyth and W. S. Walls, *J. Am. Chem. Soc.*, **54**, 2261 (1932)

¹² G. L. Lewis and C. P. Smyth, *J. Chem. Phys.*, **7**, 1085 (1939).

The agreement of this moment value 3.67 calculated for the gas with the observed⁷ value 3.69 is well within the accuracy of the values used.

The calculation of the moment of a molecule containing two or more dipoles which may change their directions relative to one another through rotation around valence bonds is based, as previously indicated, upon averaging over-all possible positions around the bonds. The method is embodied in the statement¹⁰ that the mean square of n vectors is equal to the sum of the squares of the lengths of the separate vectors plus twice all the possible products of the lengths of two vectors multiplied by the product of the cosines of the angles made by the directed lines connecting the pair. The method may be illustrated by calculating the moment value in solution of β, β' -dichlorodiethyl ether:



The carbon valence angle is 110° and the oxygen valence angle may, without serious error, be taken as 110° . The angles between the directed lines are consequently $180^\circ - 110^\circ = 70^\circ$. The molecule may be treated as containing four dipoles, two acting along the lines of the two C—Cl bonds, each having a moment $m_1 = 1.7$, the solution value for methyl chloride, and two along the lines of the two C—O bonds, each having a moment m_2 . m_2 may be calculated from the moment of dimethyl ether in table I, which is the resultant of two such dipole moments, $2m_2 \cos 55^\circ = 1.30$. Although the value $m_2 = 1.13$ thus obtained is for the gas and not for the solution, the possible error resulting from its use is no greater than the uncertainty in the m_1 value. When two dipoles point in opposite directions along the chain, a minus sign must be given to one of their moments. Thus, the product of the Cl—C and the C—O moment first reached in going along the chain from it is negative as it appears in the expression for the mean square moment obtained by application of the rule stated above:

$$\mu^2 = 2m_1^2 + 2m_2^2 - 4m_1m_2 \cos^2 70^\circ + 4m_1m_2 \cos^3 70^\circ - 2m_2^2 \cos 70^\circ - 2m_1^2 \cos^5 70^\circ$$

With the values for m_1 and m_2 given above, this equation gives a moment 2.58 as compared to the value 2.57 found for the molecule in benzene solution.¹¹ The excellence of the agreement between the calculated and the observed values is somewhat fortuitous as one might almost equally well use $m_1 = 1.8$, which would give a calculated moment 2.71. The difference 0.14 between the two calculated values is of the order of magnitude of the probable error due to group moment errors and inductive effects. Much

greater differences between calculated and observed moment values are indicative of restriction of rotation around one or more bonds.

II. CALCULATION OF MOMENT FROM DIELECTRIC CONSTANT

The dipole moment of a molecule is ordinarily obtained from measurements of the dielectric constant of the substance concerned in the gaseous state or in dilute solution in a nonpolar solvent. Because of the high precision required to obtain significant dielectric constant values for gases, the less exacting measurements on solutions are usually employed. The dielectric constant is best determined by measuring with a high-frequency alternating current the capacity of a condenser consisting of parallel plates or concentric cylinders of metal. The dielectric constant is then given by the ratio of the capacity, C , of the condenser filled with the material in question to the capacity, C_0 , of the empty condenser, or $\epsilon = C/C_0$. The dielectric constant is greater than unity because the potential difference which drives the alternating current through the condenser tends to turn the molecules, so that their permanent dipoles oppose the applied field, and, in addition, induces a small dipole in each molecule in opposition to the field. This behavior of the dielectric constant, ϵ , is expressed quantitatively by the equation of Debye:¹³

$$[(\epsilon - 1)/(\epsilon + 2)][M/d] = 4\pi N\alpha_0/3 + 4\pi N\mu^2/9kT$$

in which M is the molecular weight of the substance, d is the density, N is the number of molecules in a mole, 6.023×10^{23} , k is the gas constant per molecule, 1.38×10^{-16} , T is the absolute temperature, α_0 is the molecular polarizability, that is, the dipole moment induced in a molecule by unit electric field, and μ is the permanent dipole moment of the molecule. The equation may be abbreviated to:

$$P = a + b/T$$

in which $P = [(\epsilon - 1)/(\epsilon + 2)][M/d]$ is called the molar polarization, the constant $a = 4\pi N\alpha_0/3$ is the induced polarization, and the constant $b = 4\pi N\mu^2/9k$. Determination of the dipole moment is then a question of obtaining the value of b . The induced polarization consists of the effect of the induced shift of the electrons in the molecule, which may be termed the electronic polarization, P_E , plus a small contribution from shifts of ions, atoms, or radicals, which is called the atomic polarization, P_A . We thus have:

¹³ P. Debye, *Physik. Z.*, 13, 97 (1912).

$$a = P_E + P_A$$

A similar shift of electrons induced by the electric field of a light wave is responsible for the molar refraction of the substance for visible light:

$$R = [(n^2 - 1)/(n^2 + 2)][M/d]$$

in which n is the refractive index. Extrapolation of the molar refraction by means of a simple dispersion formula to infinite wave length, which corresponds practically to the low frequency of the electric field used in measuring the dielectric constant, gives a value of P_E about 3 or 4% lower than the molar refraction for the yellow D sodium line. As P_A , which is dependent upon characteristic vibrations in the infrared region, cannot be determined directly for practical purposes, a value equal to 15%, or more recently, 5%, of P_E is sometimes assigned to it. However, in cases where a can be reliably determined directly, the difference $a - P_E = P_A$ is usually less than 15% of P_E and is sometimes no more than 3%. It seems as well, therefore, to avoid this arbitrary assignment of an often too large value to P_A and use the value of R_D , the molar refraction for the D sodium line for a . This procedure may lead to appreciable error in the value calculated for the moment only when the latter is below 1.0×10^{-18} . When the moment is much lower than this value, a direct determination of a is desirable for accuracy. It can be measured directly by determining the dielectric constant and density of the substance in the solid state at a temperature so low that no molecular rotation can occur, but this is often difficult experimentally. It can also be determined from measurements in the vapor state as indicated below.

The polarization, P , is evidently a linear function of the reciprocal of the absolute temperature. Consequently, the measurement of ϵ and d at three or more temperatures over a range of 100° or more should establish a straight line for P plotted against $1/T$, the slope of this line giving the value of b and the intercept at $1/T = 0$ giving a . The establishment of such a line is the ideal method of obtaining the dipole moment, but, unfortunately, it can be applied only in the case of gases. The second term, b/T , of the Debye equation represents the contribution of the dipoles through their partial orientation in the externally applied electric field used in measuring the dielectric constant. As the orientation of the molecules is opposed by thermal agitation, the effect varies inversely as the absolute temperature. In the derivation of this expression, it is assumed that the molecules are oriented wholly at random in the absence of an externally applied field, which is true only in the case of gases. Although the equation is, therefore, strictly correct only in the case of gases, it can be applied as a satisfactory approximation to solutions in which the polar molecules under

investigation are widely separated from one another by intervening non-polar molecules, such as those of heptane or benzene. The effect of interaction, usually inductive, between the solute and solvent molecule, commonly referred to as the solvent effect, may change the apparent moment found for the molecule by a few per cent from the correct value which would be given by the gas. As the solvent effect changes with temperature, it leads to serious error in the slope of the $P-1/T$ line, which, therefore, cannot be obtained from measurements in solution.

The polarization of a binary mixture is given by the expression:

$$P_{1,2} = c_1 P_1 + c_2 P_2 = [(\epsilon - 1)/(\epsilon + 2)][(c_1 M_1 + c_2 M_2)/d]$$

in which c_1 and c_2 , P_1 and P_2 , and M_1 and M_2 are, respectively, the mole fractions, the molar polarizations, and the molecular weights of the individual components, 1 and 2, while ϵ and d are the dielectric constant and density of the mixture. Substitution of the relation, $c_2 = 1 - c_1$ and rearrangement give:

$$P_2 = (P_{1,2} - P_1)/c_2 + P_1$$

If P_1 is taken as the polarization of the solvent and assumed to be identical in value with that of the pure solvent and unaffected by the solute, which is not strictly true, the polarization, P_2 , of the solute may be calculated from the dielectric constant and density found for the solution. In a large majority of cases, the values of P_2 increase with decreasing concentration, the rate of increase tending to be greater the lower the concentration. The measurements are made upon four or five solutions covering concentration ranges such as $c_2 = 0.001$ to 0.01 or $c_2 = 0.02$ to 0.10 , which are selected according to the accuracy of the apparatus, the size and variability of P_2 , and possibly the solubility of the solute. The resulting values of P_2 are plotted as ordinates against those of c_2 as abscissae and the curve is extrapolated to $c_2 = 0$ to obtain a polarization value P_∞ for the solute at infinite dilution, where the solute molecules should be oriented at random in the absence of an externally applied field.

The great majority of the dipole moment values in the literature has been obtained by extrapolation of the polarizations in solution, although the number of accurately measured gas values is now considerable. An alternative method of handling the solution data is the direct extrapolation^{14,15} of the dielectric constant and the density, rather than the polarization, as previously mentioned. In the majority of cases the dielectric constants, ϵ , and the specific volumes, $v = 1/d$, of the dilute solutions are found¹⁴ to be linear functions of the weight fractions, w_2 , of the solute, that is:

¹⁴ G. Hedestrand, *Z. physik. Chem.*, **B2**, 428 (1929).

¹⁵ I. F. Halverstadt and W. D. Kumler, *J. Am. Chem. Soc.*, **64**, 2988 (1942).

$$\epsilon = \epsilon_1 + \alpha w_2 \text{ and } v = v_1 + \beta w_2$$

where ϵ_1 and v_1 are the dielectric constant and the specific volume of the solvent, respectively, and α and β are the slopes of the straight lines obtained by plotting ϵ and v against w_2 . Halverstadt and Kumler¹⁵ have derived from these relations an equation for the solute polarization at infinite dilution which may be written as:

$$P_{\infty} = \frac{3\alpha v_1 M_2}{(\epsilon_1 + 2)^2} + M_2(v_1 + \beta) \frac{(\epsilon_1 - 1)}{(\epsilon_1 + 2)}$$

When ϵ is plotted against w_2 , the value of ϵ_1 is given by the intercept at $w_2 = 0$ and the value of α by the slope of the line. The values of v_1 and β are obtained in similar manner. The values of these constants may also be obtained from the experimental data by the method of least squares. If, as occasionally may happen, the curves for ϵ and v are not linear, their equations become quadratic and cumbersome to handle. The method should then be abandoned or applied only to such dilute solutions as may conform to a linear relation. As compared to the previously described method of plotting P_2 against c_2 and extrapolating to $c_2 = 0$ to obtain P_{∞} , this method has the advantage that the necessary extrapolations are linear. It has an advantage also in that it reduces the error occasionally caused by contamination of the solutions, as, for example, by absorption of water vapor, with consequent change in the dielectric constant of the solvent. Any considerable difference between the ϵ_1 obtained by the linear extrapolation and that obtained by direct measurement on the pure solvent may be taken as an indication of contamination of the solutions, the damaging effect of which is minimized by use of the extrapolated value for ϵ_1 . This method and the polarization extrapolation method lead to similar results and both may be employed with profit, particularly when the experimental errors are large or the solutions very dilute.

P_{∞} is usually in error because of the solvent effect, which, depending on the molecular forms, the locations of the dipoles, and the polarizabilities involved, may be negligibly small or may affect the moment value by as much as 10%, commonly lowering it.¹⁶⁻¹⁹ In view of this solvent effect, it is obviously needless to worry over the usually much smaller uncertainty resulting from ignorance of the atomic polarization, P_A . The dipole part of the polarization can, therefore, be obtained by subtracting the molar refraction, R_D , for the D sodium line from the value of P_{∞} and used

¹⁶ F. H. Müller, *Physik. Z.*, **34**, 689 (1933).

¹⁷ F. C. Frank, *Proc. Roy. Soc. London*, **A152**, 171 (1935).

¹⁸ K. Higasi, *Sci. Papers Inst. Phys. Chem. Research (Tokyo)*, **28**, 284 (1936).

¹⁹ W. P. Conner, R. P. Clarke and C. P. Smyth, *J. Am. Chem. Soc.*, **64**, 1379 (1942)

to calculate the moment according to the following equation, in which the universal constants of the Debye equation are replaced by numerical values:

$$\mu = 0.01281 \times 10^{-18} [(P_{\infty} - R_D)T]^{1/2}$$

The method of calculation and the character of the data obtained with the pycnometer mentioned and the heterodyne beat apparatus described in detail subsequently in this chapter (page 1634) are illustrated by the measurements in table II carried out by Dr. Peter F. Oesper in the deter-

TABLE II
DATA AT 25° C. FOR SOLUTIONS OF 2-NITROPROPANE ($M_2 = 89.10$)
IN BENZENE ($M_1 = 78.11$)

Solu- tion No.	Solute weight, g.	Moles solute	Benzene weight, g.	Moles benzene	Total moles	Mole fraction solute, c_2	Mole fraction benzene, c_1
1	0	(Solution 1 is the pure solvent)				0	1
2	0.2976	0.003340	31.322	0.40099	0.40433	0.008261	0.99174
3	0.4956	0.005562	28.864	0.36953	0.37509	0.014828	0.98517
4	0.9708	0.010895	27.074	0.34661	0.35751	0.030475	0.96953
5	2.0711	0.023243	26.961	0.34517	0.36841	0.063090	0.93691

Solu- tion No.	$c_1 M_1 +$ $c_2 M_2$	ϵ	d	$P_{1,2}$	$c_1 P_1$	$c_2 P_2$	P_2
1	78.11	2.2773	0.87219	26.743 ($= P_1$)			
2	78.201	2.4070	0.87317	28.593	26.522	2.071	251
3	78.273	2.5135	0.87391	30.034	26.346	3.688	249
4	78.445	2.7681	0.87575	33.215	25.928	7.287	239
5	78.803	3.3121	0.87950	38.998	25.056	13.942	221

mination of the moment of 2-nitropropane in benzene solution. Extrapolation of the P_2 values to $c_2 = 0$ gives $P_{\infty} = 258 \pm 2$; $R_D = 21.6$; and $\mu = 0.01281 \times 10^{-18} [(258 - 21.6) \times 298]^{1/2} = (3.40 \pm 0.02) \times 10^{-18}$.

Although it is evident that the absolute value of a dipole moment obtained from measurements in solution may, however precise the measurements, be in error by as much as 10%, the errors are usually smaller and are similar for similar substances in the same or in similar solvents. Differences as small as 2% between values for different molecules obtained under similar conditions may have some significance, although they should be treated with discretion. Various simple formulas, empirical and otherwise, have been used to calculate the solvent effect with considerable success for some classes of substances, and with failure for others. The following approximate equation has been developed¹⁹ for the ratio of the moment in solution to that of the gas on a theoretical basis and has been applied successfully to a wide variety of substances:

$$\mu_{\text{soln.}}/\mu_{\text{gas}} = 1 + 0.43A (\epsilon - 1)$$

in which ϵ is the dielectric constant of the solvent and A is a constant, usually negative, characteristic of the solute and dependent upon the shape of its molecule and the location of the dipole in it.¹⁸ Division of the second term on the right by a factor, 0.14 ($\epsilon + 2$), would give an equation identical with that developed by Higasi.¹⁸ As the equation can be written in the form:

$$\mu_{\text{soln.}} = \mu_{\text{gas}} + 0.43A \mu_{\text{gas}}(\epsilon - 1)$$

it is evident that a value can be obtained for the moment in the gaseous state by extrapolating to $\epsilon = 1$ the straight line obtained by plotting as ordinates the values of the moments obtained in two or more different solvents against the values of ϵ for the solvents as abscissae. The value thus obtained, although considerably less accurate than a good direct determination in the gaseous state, has greater absolute accuracy than a value determined in a single solvent. For use in rough structural considerations or more careful comparison of closely related molecules, an adequate moment value can accordingly be obtained from the dielectric constants and densities of three or four different solutions in one solvent and the molar refraction. As indicated above, better values are obtainable by the use of several solvents. For the quantitative consideration of small differences in moment, measurements in the gaseous state are desirable though much more difficult.

The necessity of using gas or dilute solution measurements for the calculation of dipole moment by means of the Debye equation arises from the fact that the latter does not take adequate account of the effects of the electric fields of polar molecules upon their immediate neighbors. A better approximation, but still an approximation, was developed by Onsager²⁰ and used by Böttcher²¹ for the calculation of the dipole moment from data for the pure polar liquid by means of an equation which may be written in the following form:

$$\mu^2 = \frac{9MkT}{4\pi Nd} \frac{(\epsilon - n^2)(2\epsilon + n^2)}{\epsilon(n^2 + 2)^2}$$

The refractive index, n , used by Böttcher was the value, n_∞ , for infinite wave length obtained by the use of the dispersion formula:

$$R_\infty = \frac{\lambda_2^2 - \lambda_1^2}{(\lambda_2^2/R_2) - (\lambda_1^2/R_1)}$$

where R is the previously defined molar refraction, R_1 , measured at wave

²⁰ L. Onsager, *J. Am. Chem. Soc.*, **58**, 1486 (1936).

²¹ C. J. F. Böttcher, *Physica*, **6**, 59 (1939).

length λ_1 ; R_2 is measured at wave length λ_2 ; and $R_\infty = [(n_\infty^2 - 1)/(n_\infty^2 + 2)][M/d]$, calculated from the above equation. Since n^2 calculated in this way contains no contribution from atomic polarization, it would seem better, as well as easier, to use for n the refractive index n_D measured for the D sodium line, thus making, at least, a partial allowance for atomic polarization, as was done when R_D was subtracted from P_∞ for the calculation of the moment. Except for small moment values, the effect of atomic polarization would, in any event, be negligible in this calculation. Although this calculation of the moment directly from the dielectric constant, density, and refractive index of the pure polar liquid is an approximation and has not yet been widely applied, Böttcher has obtained with it values for a number of moments which are closer to the correct gas values than those given by solution measurements. For some liquids, at least, the error involved in the calculation would appear to be less than the solvent effect. For the ethers and, particularly, the alcohols, the moment values obtained by this method are considerably higher than the gas values. For ethylene chloride, the moment value obtained is close to that calculated for unrestricted rotation around the C—C bond, a value much higher than that found in the vapor state and in most solutions and, probably, close to the true value in the pure liquid.

The results obtained by the different methods may be illustrated by the values for the often-measured substance, nitrobenzene, seemingly undependable values being omitted. Three different sets of measurements for the moment of the molecule in the vapor state give 4.19, 4.23, and 4.24. A large number of measurements in various solvents give values from 3.84 to 4.10 calculated by extrapolation of the polarization. Four sets of measurements in benzene give, by polarization extrapolation, 3.97, 4.08, 3.93, and 3.94, and, by dielectric constant and density extrapolation,¹⁵ 3.98, 3.98, 4.00, and 4.01, respectively. Calculation by Böttcher from the dielectric constants and the densities of the pure liquid at temperatures ranging from the freezing point almost to the boiling point gives values varying from 4.09 to 4.26 with a mean of 4.2, in close agreement with the gas value. The dielectric constant and density extrapolation method reduces considerably the discrepancies among the four values calculated by the polarization extrapolation method and gives a mean value 3.99, in close agreement with the solution value 3.98 in table I, which was obtained as an average of the best values given by the polarization extrapolation method.

The polarization extrapolation method is the standard method which has been most generally used for calculating dipole moments from measurements on solutions, but the dielectric constant and density extrapolation method provides a useful and, sometimes, preferable alternative, whose results may profitably be checked against those of the other method. The

calculation from data for the pure liquid may give a value closer to the gas value than is the solution value, but cannot always be depended upon.

III. REFRACTION

The molar refraction, R , can often be found in the tables in Landolt-Börnstein (5th ed.) or calculated from the refractive indices and densities in *International Critical Tables*. Unless there is a conjugated system in the molecule with consequent exaltation of the refraction, the molar refractions of most organic molecules can be calculated with sufficient accuracy for the moment calculation from the table of atomic refractions on page 1163 or from the table of electron group refractions on p. 1164. The refractive index is easily measured with either an Abbe refractometer or the more accurate Pulfrich instrument, the light source being a sodium flame or, better, a sodium vapor lamp, or a small hydrogen discharge tube if other wave lengths are desired (compare Chapter XX, page 1141). As the molar refraction is, for our purposes, practically independent of concentration, the value for the pure substance is used. If the pure substance is a solid, its molar refraction can be obtained by measuring the refractive index and density of a solution and applying equations exactly analogous to those used for the polarization, P :

$$R_{1,2} = [(n^2 - 1)/(n^2 + 2)][(c_1M_1 + c_2M_2)/d]$$
$$R_2 = (R_{1,2} - R_1)/c_2 + R_1$$

in which R_1 and R_2 are the molar refractions of the solvent and the solute, respectively.

IV. DENSITY

The density may be measured most simply with a pycnometer fitted with ground-glass caps to prevent loss by evaporation, an accuracy of 2 units in the fourth decimal place being ample for the more concentrated solutions. For concentrations below $c_2 = 0.01$, the error should be reduced to as few units as possible in the fifth decimal place. In the neighborhood of $c_2 = 0.001$ and below, it may prove desirable to obtain the density by extrapolating a density-concentration curve from a region of concentration at which the values are accurate. See also Chapter VI, page 253.

V. METHODS OF MEASUREMENT OF DIELECTRIC CONSTANT

It should be mentioned at this point that the terms "capacity" and "capacitance" and "condenser" and "capacitor" are used interchangeably in this chapter as they are in the literature. Except when microwaves are

employed, the dielectric constant is normally determined by the measurement of the electric capacity. A conductor charged with a quantity of electricity q to a potential V has a capacity $C = q/V$. A typical simple condenser consists of a pair of conducting plates, each of area A cm.² with a distance d cm. between them and a capacity $C = \epsilon A/4\pi d$ electrostatic units = $0.0885\epsilon A/d$ micromicrofarad, where ϵ is the dielectric constant of the medium between the plates. Although, in the m.k.s. system commonly employed in engineering practice at the present time, the dielectric constant of free space $\epsilon_0 = 8.854 \times 10^{-12}$ farad meter⁻¹, the dielectric constant is normally obtained as a ratio and is thus a pure number. It is given experimentally by the previously mentioned relation, $C = \epsilon C_0$, where C_0 is the capacity of a condenser with a vacuum between the plates, and C is the capacity when the space between the plates is filled with material of dielectric constant ϵ . For the measurement of liquids, it is customary to take the capacity of the condenser filled with air as C_0 , since the dielectric constant of air at 0° C. and 760 mm. is only 1.00058.

The two principal methods of measuring the capacity of a condenser and, hence, the dielectric constant of a substance filling the condenser, are the capacity bridge method and the method of adjusting the capacity of a circuit until the alternating current flowing in the circuit has a certain characteristic or desired frequency. The bridge method, which, in principle, is familiar to the chemist through its use in conductance measurements, is capable of the accuracy required for any ordinary measurements on liquids or solids and has even been applied occasionally to gases. It is the most generally applicable method, since a properly designed and carefully used bridge may be employed on solutions with specific conductivities as high as 10^{-4} ohms⁻¹ cm.⁻¹, while the frequency methods often become undependable for conductances above 10^{-8} . Because an increasing number of investigators are becoming interested in the dielectric constants of protein solutions, which have considerable conductances, a brief outline of the bridge method will be given together with references to papers in which experimental details are available. Because of its use by many investigators, a very brief outline will also be given of the resonance method. The heterodyne beat frequency method gives the greatest accuracy when conductance of the material to be measured is not a disturbing factor. Since a very large majority of the solutions to be measured in dipole moment determinations have conductances far too low to affect the accuracy of the heterodyne beat method and, since a maximum of precision is obtainable by this method with a minimum of effort, it will be described in detail. An account of various methods has been given by Hartshorn.²²

²² L. Hartshorn, *Radio-frequency Measurements by Bridge and Resonance Methods*. Wiley, New York, 1941.

1. Bridge Method

The bridge method for measuring capacities is closely analogous to that for measuring conductances described in Chapter XXV, page 1651. In this, and in the resonance and heterodyne beat methods, the replacement or substitution method of balancing is generally desirable, that is, balance is obtained by adjustment of a variable precision condenser, and the cell or condenser to be measured is connected in parallel with the precision condenser, which is then turned down until a balance is again obtained. The difference between the two condenser readings gives the capacity to be measured.

The balance of the bridge is obtained by reducing to zero the voltage between two opposite corners, a condition which occurs when the ratio of the impedances of two adjacent bridge arms is equal to the ratio of the impedances of the other pair of arms. The capacitance bridges normally used for the measurement of dielectric constants contain two resistance arms, the so-called ratio arms, and two capacitance arms. In such bridges inductance is kept to a minimum and, by symmetrical construction of the bridge parts, is made to cancel out. Since, however, the material to be measured may have appreciable conductance, and since small inequalities in resistance may occur elsewhere in the system, small noninductively wound resistances are placed in series with the capacitances or large non-inductively wound resistances are placed in parallel with them. The large parallel resistances, of the order of 10,000 ohms, have been found more satisfactory in the bridge used in the author's laboratory. In balancing the bridge, capacitances and resistances are adjusted alternately until a minimum current is obtained in the detector across the corners of the bridge. If the conductance of the sample to be measured is negligible, a single adjustment of the capacitance is all that is necessary. If R_1 is the resistance in parallel with the precision measuring condenser and the measuring cell of resistance R_m , and R_2 is the resistance in parallel with the other capacitance arm of the bridge, at balance: $1/R_m = 1/R_2 - 1/R_1$. The specific conductance, k , of the dielectric under measurement in the cell is then: $k = 0.0885/C_0 R_m$, where C_0 is the geometrical capacitance (in micromicrofarads) of the empty cell. The loss factor, or imaginary part, ϵ'' , of the dielectric constant of the material under measurement can be calculated as $\epsilon'' = 1.8 \times 10^{12} k/f$, where f is the frequency.

The proper shielding of the bridge is of great importance and depends in detail upon the particular type of bridge. In general, it is essential that the shielding eliminate undesirable capacitive and inductive coupling, any stray pickup, and any uncontrolled variation in capacitance, for example, such as might occur between any portion of a circuit not at earth potential and the body of the observer. Bridges are, therefore, completely sur-

rounded by a grounded shield and the shafts on all controls are either grounded or insulated. The ground connection is commonly a wire soldered to a water pipe. The analysis of bridge networks involved in the complete treatment of shielding is beyond the scope of this chapter and is treated in detail elsewhere.^{22, 23} Discussions of the principles of shielding are to be found in many handbooks and textbooks.^{24, 25}

It is usually preferable to purchase a bridge rather than devote time and money to the construction of an apparatus which may be unsuccessful. From the point of view of dipole moment measurements, the objection to many commercially available bridges is that they are designed for the measurement of capacities up to many microfarads, whereas the cells used in the determination of dipole moment rarely exceed a few hundred micromicrofarads in capacity when filled with the material to be measured. Many commercial bridges are so designed that the capacitance to be measured forms an entire arm of the bridge. The absolute accuracy of such an instrument can often be greatly improved by substituting a precision measuring condenser in this arm of the bridge, balancing the bridge, attaching the cell to be measured in parallel with the precision condenser, and turning down the latter until the bridge is balanced again. The difference between the two condenser readings usually gives the unknown capacitance with an accuracy unobtainable with an instrument in another arm of the bridge. In the selection or construction of a bridge, an error in measurement less than 0.2 micromicrofarad should be sought.

Oncley²⁶ has described the use of the radio-frequency bridge type 516-C, manufactured by the *General Radio Company*, Cambridge, Mass., over a range of 25 to 2500 kilocycles per second on solutions with specific conductances up to 10^{-4} mhos cm.⁻¹ but this instrument has recently been withdrawn from manufacture. The use of the Schering bridge, which is capable of great accuracy when properly used, has been described briefly by Fuoss.²⁷ In the Schering bridge, the elementary circuit of which is shown in figure 1, the simplified conditions of balance are given by:

$$C_x = C_N(R_A/R_B)$$

$$D_x = 2\pi f R_x C_x = 2\pi f R_A C_A$$

where D_x , the dissipation factor, is the ratio of resistance to reactance. In the type 716-C Schering-type capacitance bridge manufactured by *General*

²² B. Hague, *Alternating Current Bridge Methods*. Pitman, London, 1938.

²⁴ F. E. Terman, *Radio Engineers Handbook*. McGraw-Hill, New York, 1943, pp. 128-134.

²⁵ E. E. Zepler, *The Technique of Radio Design*. Wiley, New York, 1943, pp. 183-205.

²⁶ J. L. Oncley, *J. Am. Chem. Soc.*, **60**, 1115 (1938).

²⁷ R. M. Fuoss, *J. Am. Chem. Soc.*, **59**, 1703 (1937).

Radio Company, the capacitance, C_A , consists of an air condenser and a mica decade condenser connected in parallel by suitable switching. The standard condenser, C_N , is calibrated directly in micromicrofarads from about 100 to 1100 $\mu\text{mf.}$ and the total capacitance range of the bridge is extended to 0.011, 0.11, and 1.1 $\mu\text{f.}$ by changing the resistance, R_B , to 2000, 200, and 20 ohms, respectively. In a dipole moment measurement, the capacitance of the cell can be accurately measured by placing a precision condenser across the "unknown" terminals of the bridge, adjusting the condenser to balance, connecting the cell in parallel with the precision condenser, and turning down the latter until the bridge is balanced again.

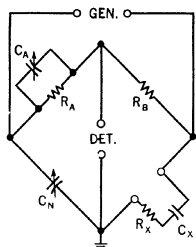


Fig. 1. — Schering bridge.

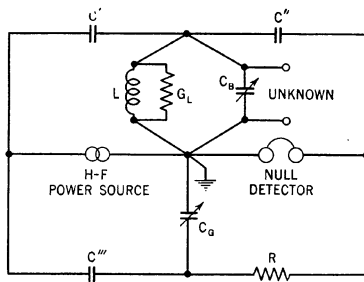


Fig. 2.—Twin-T impedance measuring circuit.

(Courtesy General Radio Company)

The difference between the two precision condenser readings can give the capacitance of the cell with a precision of 0.1 $\mu\text{mf.}$ An oscillator, amplifier, and detector must be attached to the bridge. For audio frequencies, a General Radio Company type 1301-A oscillator or a type 913-B beat-frequency oscillator may be used as power source. Type 1231-A amplifier and null detector is recommended for use as the detector. A Western Electric 1002-C telephone can be used with the amplifier as a null indicator. The type 707-A cathode-ray null detector also can be used for frequencies up to 2 kilocycles. Although the calibration adjustments of the bridge are made at 1 kilocycle, it may be used at frequencies from 30 cycles to 300 kilocycles. The theory of bridge measurements has been treated extensively by Hague.²³

In place of the conventional bridge, the "Twin-T" instrument developed²⁸ by *General Radio Company* may be used¹⁹ advantageously for measurements at frequencies from 0.46 to 40 megacycles. This instrument

²⁸ D. B. Sinclair, *Proc. Inst. Radio Engrs.*, 28, 310 (1940).

is particularly useful for measurements on weakly conducting solutions with specific conductances below 10^{-4} mho cm. $^{-1}$, for which the heterodyne beat method and many bridges cannot be used. Because of the magnitude of the frequency in the upper range, inductive effects may cause very large errors unless proper precautions are observed. For this reason, the ungrounded lead of the measuring cell used with this apparatus in the writer's laboratory was reduced to a banana plug.¹⁹ Because of the possibility of serious inductive errors, a lower frequency is preferable in the absence of appreciable conductance. The instrument uses a parallel-T null circuit as shown in the schematic diagram in figure 2. An initial balance of the circuit is obtained with the unknown or measuring cell disconnected, the precision condenser of C_B set at a convenient value, C_1 , and the conductance dial of C_G set at zero. A minimum in the background noise or in the beat note from the beat frequency oscillator of the receiver may be taken as the balance point, which is obtained by adjustment of auxiliary condensers. The cell is then connected and the circuit rebalanced by adjusting C_B and C_G . In the absence of appreciable inductance effects, the difference between the capacitor reading C_1 and the reading C_2 when the circuit is balanced with the cell connected gives the capacitance of the cell in micromicrofarads. The conductance is given by:

$$G_x = \omega^2 C' C'' \Delta C_G / C''' = k \omega^2 \Delta C_G$$

and the dial of C_G is calibrated to be direct reading at 1, 3, 10, and 30 megacycles. In most dipole moment measurements the conductance is negligibly small.

In the higher part of the frequency range, the inductive effects in the cell and its leads and in the precision condenser itself may be eliminated by use of the following equations:¹⁹

$$C_x = C_1 - C_2 + \Delta C_1 + \Delta C_2 + \Delta C_3$$

$$\Delta C_1 = \frac{\omega^2 L_c C_1^2}{1 - \omega^2 L_c C_1} - \frac{\omega^2 L_c C_2^2}{1 - \omega^2 L_c C_2}$$

$$\Delta C_2 = - \frac{\omega^2 L_x C_x^2}{1 - \omega^2 L_x C_x}$$

$$\Delta C_3 = L_x (G_x')^2$$

$$G_x' = G'' - 2\omega L'' G'' C_1 + R_c \omega^2 (C_2^2 - C_1^2)$$

where: C_x = capacitance of cell in farads, $\omega = 2\pi \times$ frequency, L_c = inductance of the precision condenser, L_x = inductance of the cell and leads, G_x' = conductance of cell in mhos, G'' = apparent conductance of cell in

mhos as read from the conductance dial, L'' = inductance of the conductance condenser, and R_c = resistance of the leads to the conductance condenser in ohms. When the frequency and inductance are sufficiently high to necessitate the use of these corrections to the capacity difference read directly from the scale of C_B , the values for the various constants of the apparatus are measured once and for all, C_1 is kept at a convenient fixed value, and graphical methods may be used to facilitate the calculation of the corrections. Corrected values of 300 $\mu\mu\text{f.}$ may be obtained for C_2 with an accuracy slightly better than $\pm 0.1 \mu\mu\text{f.}$

A suitable radio-frequency generator and detector should be connected to the "Twin-T" circuit by means of coaxial cables. The type 805-C standard signal generator is suitable as a power source, as are the older type 605-B and the type 684-A modulated oscillator manufactured by *General Radio Company*. A well-shielded radio receiver, such as the Hallcrafters "Super Defiant," may be advantageously used for the detector.

2. Resonance Method

In the resonance method, a high-frequency alternating current is set up in a primary circuit which is loosely coupled to a secondary circuit containing capacity and inductance. The current flowing in this secondary circuit can be altered by change in the capacity or the inductance of the circuit, a maximum value being reached when the product of the capacity, C , and the inductance, L , is equal to the reciprocal of the square of 2π times the frequency, f , of the current, or $f = 1/2\pi\sqrt{LC}$, a condition called resonance. Resonance is obtained by adjusting the capacity of a precision condenser in the secondary circuit until the current reaches a maximum, as shown by a detector circuit coupled to it. When this adjustment has been made, the cell to be measured is connected in parallel with the precision condenser, which is lowered until the detector again shows a maximum current. The difference between the two condenser readings gives the capacity of the cell. The method has been used rather widely upon liquids, and is of such accuracy that it has been applied by Groves and Sugden²⁹ to measurements of gases. An apparatus in which a very small resonator is completely immersed in the liquid to be measured has been used by Wyman³⁰ to obtain accurate values for solutions having conductances up to about 100 times that of water.

A resonance apparatus developed by Henriquez³¹ was manufactured by *Kipp & Zonen*, Delft, Holland. For not very accurate measurement of

²⁹ L. G. Groves and S. Sugden, *J. Chem. Soc.*, 1934, 1094.

³⁰ J. Wyman, *Phys. Rev.*, 35, 623 (1930).

³¹ P. C. Henriquez and A. W. Renaud, *Physica*, 2, 429 (1935).

the dielectric constant of a material, an apparatus called an "elmometer" was furnished, while a "precision apparatus for the measurement of dielectric constants (dipole measurements)" was provided for more accurate measurements. The measurement is made in a high-frequency oscillating circuit which is in resonance with a quartz crystal. The plate current of an oscillator in the circuit varies very rapidly with the capacitance in a certain region. Below this region of capacitance, increase of the capacitance in the anode circuit causes the plate current to fall with gradually increasing rapidity until it reaches a minimum, beyond which a very small increase in capacitance causes a sharp rise in current. A milliammeter reading part way up this sharp rise in current corresponds to an exactly fixed capacitance in the circuit, which is read on a precision condenser. The connection of a measuring cell in parallel with the precision condenser or the filling of a cell already in parallel with the condenser is followed by the downward adjustment of the precision condenser until the milliammeter reading is restored to its original value. The difference between the two condenser readings gives the capacitance of the cell or the increase in its capacitance caused by the addition of the sample. In order to check the constancy of the circuit during a measurement, a reference condenser mounted in the apparatus may be switched in and out for balance before and after the measurement of the cell. A high degree of precision has been claimed for this apparatus. A similar method has been described by Le Fèvre.³²

A modernized version of this method has recently been described,^{33, 34} in which the oscillator circuit (Fig. 3) is controlled by a quartz crystal and an electron ray tube 6E5 serves both as oscillator tube and as resonance detector. When the capacitance C in the plate-tuning circuit is turned from low capacitance up, the condition of resonance is reached and oscillation sets in when the natural frequency of the circuit approaches that of the crystal. The setting in of oscillation is accompanied by a reduction in plate current. If the capacity C is further increased, the plate-tuning circuit is no longer in resonance with the crystal, oscillation ceases abruptly, a sudden jump in plate current occurs and the shadow angle of the "tuning eye" of the tube suddenly increases. The increase in the capacitance C through the resonance point is accompanied by gradual decrease of the shadow angle to a minimum, followed by sudden widening out as the resonance point is passed and oscillation ceases. The critical capacity setting sharply determined by the sudden widening of the shadow angle is taken as

³² R. J. W. Le Fèvre, *Dipole Moments; Their Measurement and Application*. Chemical Pub. Co., New York, 1938, Chapter 2. 2nd ed., Methuen, London, 1948.

³³ F. C. Alexander, Jr., *Electronics*, 18, No. 4, 116 (1945).

³⁴ P. Bender, *J. Chem. Education*, 23, 179 (1946).

accuracy of one in the third decimal place is claimed in the measurement of small dielectric constant values such as those observed in dipole moment determinations. The apparatus can be made automatic and recording by connecting an spdt relay in the plate circuit of the 6E5 tube and using a suitable recording device. The relay operates a reversible 4-r.p.m. motor connected to the vernier drive of the dial of the measuring condenser.

3. Heterodyne Beat Method

This method has been previously described by the writer in considerable detail,³⁵ but, as recent developments in vacuum tubes have made improvements possible, a relatively new apparatus will be described here. Although the wiring diagram is necessarily complicated, the method is essentially simple in principle and capable of any accuracy attainable with the precision condenser. It depends upon the control of the frequency of a vacuum-tube oscillator by adjustment of the capacity of its circuits, the resistance and inductance remaining constant. Essentially, the apparatus consists of two separate oscillators, one of which is now usually held at constant frequency by the vibration of a quartz crystal while the other is adjusted by varying its capacity to give a frequency different by a constant amount or by zero from that of the fixed oscillator. The frequencies of the oscillators are far above the audible range, but the difference between them is a frequency audible as a distinct musical note in a telephone connected to an amplifier to which the two oscillators are coupled. When the variable oscillator is adjusted so that its frequency is very close to, or identical with, the frequency of the other oscillator, the audible beats between the two disappear and the phone is silent. The region of silence can be made so narrow as to extend over a capacity change within the accuracy of setting of an ordinary precision condenser.³⁵ This is sufficient for the determination of the dielectric constants of most solutions down to a concentration of 0.01 mole fraction of solute and often of still more dilute solutions. However, the precision of the frequency balance can very easily be increased by the addition to the apparatus of a 1000-cycle tuning fork oscillator such as is used as a source of current for conductivity measurements. The variable oscillator is adjusted to give a difference of about 1000 cycles from the fixed oscillator. If this difference is, say, 1001 cycles, a slow throbbing beat, one per second, is heard between this note and the 1000-cycle note of the tuning fork oscillator. Further adjustment of the capacity brings the difference to 1000 cycles and eliminates the one-per-second beat. This frequency setting is considerably better than one

³⁵ C. P. Smyth, *Dielectric Constant and Molecular Structure*. Chemical Catalog Co., New York, 1931, Chapter 3.

per second, which means, if the frequency of the variable oscillator is a million cycles, an accuracy greater than one part in a million. In order to obtain the necessary delicacy in the precision condenser setting, a special small precision condenser with a long scale must be constructed, or an ordinary precision condenser must be used in series with a smaller fixed condenser. Although this added accuracy is unnecessary for the ordinary run of dipole moment determinations in solution, and is really necessary only for extremely dilute solutions and for gases, the tuning fork oscillator is included because such inclusion involves very little additional labor. Occasionally, the fixed or crystal oscillator is replaced by a pickup from some radio station.³⁶ Accounts of apparatus without any tuning fork have been given elsewhere.^{35, 36}

The apparatus to be described in detail here is one that has been described by Stranathan,³⁷ and has been used in much the same form in the writer's laboratory.³⁸ It has great precision and so much power that there is no straining of the senses to obtain accurate readings, and requires no batteries, although 6 volts d. c. have been employed for the tuning fork oscillator in the writer's apparatus, a source of such voltage being readily available in the average laboratory and a slight simplification of equipment resulting from its use. Stranathan's apparatus is mounted in portable form in a cabinet $29 \times 16 \times 13$ inches, but the apparatus used in the writer's laboratory is mounted in two boxes separated a few inches from each other, ease of portability being sacrificed for greater accessibility of the different parts. The boxes are made of $\frac{3}{4}$ -inch planks covered with stiff, galvanized iron nailed to the planks at frequent intervals to give rigidity. The sheets of galvanized iron are soldered together at least enough to give good electrical contact, and are connected to a water pipe by a soldered lead, thus forming a grounded shield. The tubes, condensers, transformers, etc., necessary for the oscillators and the amplifier may be purchased from a radio store or radio supply company at prices very low compared with those of most scientific apparatus. Because of the many different wires used, it is desirable to employ several different colors of insulation to aid in distinguishing the connections. No. 18 wire is used extensively in the connections. The apparatus used in the writer's laboratory is shown in figure 4, in which the dashed lines represent the necessary shielding of the circuits. The switches marked by short arrows, the knobs of the variable resistances forming the tuning fork volume control, P_1 , and the amplifier volume control, P_2 , and the binding posts or

³⁶ F. Daniels, J. H. Mathews, and J. W. Williams, *Experimental Physical Chemistry*, McGraw-Hill, New York, 1941, Chapter 13.

³⁷ J. Stranathan, *Rev. Sci. Instruments*, **5**, 334 (1934).

³⁸ G. L. Lewis and C. P. Smyth, *J. Chem. Phys.*, **7**, 1085 (1939).

mercury cups for connecting the cell may be mounted on a hard rubber panel forming one side of the cabinet or may be brought out through small holes in the galvanized-iron covered boxes if the latter are used as in figure 4. A hard-rubber rod attached to the screw of precision condenser C_P extends through a bushing mounted in the wall of the box, and a small

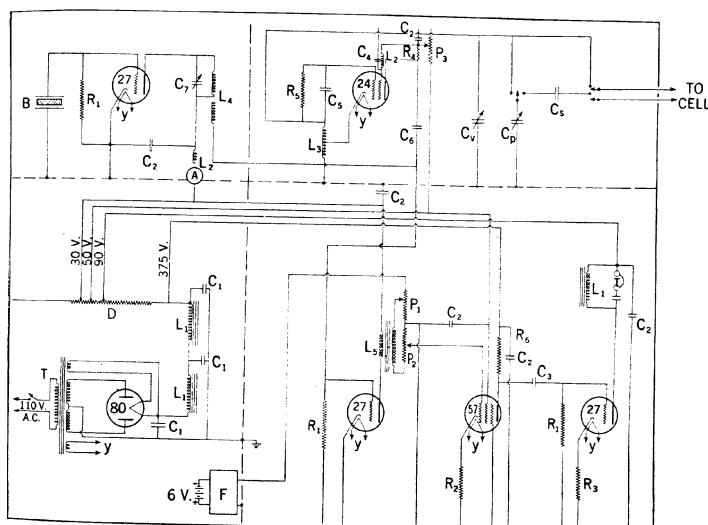


Fig. 4.—Heterodyne beat apparatus for dielectric constant measurement.

A = millimeter. B = mounted quartz crystal. D = type-D Electrad Truvolt 10,000-ohm resistor. T = Thordarson T-6912 high-power transformer. F = 1000-cycle tuning fork hummer. $R_1 = 10^6$ ohms. $R_2 = 1360$. $R_3 = 1800$. $R_4 = R_6 = 10^6$. $R_5 = 25,000$. $P_1 = P_3 = 25,000$. $P_2 = 500,000$. $C_1 = 8$ microfarads. $C_2 = 2$. $C_3 = 0.0025$. $C_4 = 0.005$. $C_5 = 0.001$. $C_6 = 5 \mu\text{f}$. $C_7 = 100$. $C_P = 1100$ (variable precision condenser). $C_V = 1000$ (variable auxiliary condenser). $C_S = 100$. $L_1 = 30$ -henry choke. L_2 = radio frequency choke. $L_3 = 51$ turns of No. 26 wire wound on an octahedral form 3.6 cm. in diameter, of which 20 turns are in the grid circuit. $L_4 = 92$ turns of No. 26 wire on a similar form in the plate circuit and 12 turns below them as the pickup. L_5 = audiotransformer with a 1:6 ratio.

hole permits the reading of the condenser scale from outside. The telephone receiver, T , may be mounted on the back of the panel behind a number of small holes, or, if so desired, brought outside the case. Shielded wire is used for the pickup leads to the oscillators.

Use of a cathode-ray oscilloscope, as employed by Dr. W. Heston in the writer's laboratory, instead of the telephone as an indicating device, in-

creases the ease with which the balance point may be obtained. The output of the amplifier is connected to the horizontal plates of the oscilloscope while the output of the 1000-cycle tuning fork oscillator is connected to the vertical plates. This 1000-cycle voltage serves as a time base for the horizontal sweep of the oscilloscope. When the precision condenser is adjusted so as to produce a 1000-cycle difference between the output of the crystal-controlled oscillator and the variable oscillator, a circular pattern will appear on the face of the cathode-ray tube. This procedure will give two different balance settings corresponding to 1000 cycles above, and 1000 cycles below, the frequency of the crystal-controlled oscillator. However, approach to the balance point from the same direction will eliminate any confusion. This visual method is less tiring and more rapid than use of the headphones described above. An electric eye tube (6E5) may be used in place of the cathode-ray oscilloscope. The 1000-cycle tuning fork oscillator will continue to serve as a time base by connecting its output to the cathode of the tuning eye tube. The output of the amplifier is fed to the grid of this tube. The shadow seen on the face of the electric eye tube will continue to open and close until the balance point has been reached; then it will remain stationary. Two balance points will also be obtained with this visual method. However, as stated previously, approach to the balance point from the same direction will eliminate any confusion. In general this null device will find application whenever an oscilloscope is not readily available.

All the tubes may be run from a common power supply, but in Strathan's apparatus the filament of tube 24 of the variable oscillator was heated from the second power supply, which was required to operate tuning fork *F*. In the apparatus employed in the writer's laboratory, the tuning fork was operated from an external source of 6 volts d. c. and all the tubes were supplied from a single transformer and type-80 rectifying tube, 30, 50, 90, and 375 volts being tapped off from a single resistor. In figure 4 the 2.5-volt leads marked *Y* on the power supply at the left of the diagram are actually connected to the similarly marked terminals of the five amplifier or oscillator tubes, one of the latter of which is controlled by the mounted quartz crystal, *B*. The actual leads connecting to *Y* are omitted from the diagram.

The necessary capacities, inductances, and resistances in the different parts of the apparatus are given under figure 4, and the voltages on the different leads are shown on the diagram. A thermostat around tube 24 is desirable when the apparatus is to be used for gases. It consists of a heavy copper cylinder 6 inches high, 4 inches inside diameter, and $\frac{1}{4}$ inch thick, wrapped with asbestos and wound noninductively with a heating coil, the top and bottom also being insulated with asbestos. It should

be maintained at approximately 40° C. by control of the current through the heating coil by means of a mercury regulator and relay.

In the single power supply used in the apparatus, 110 volts a. c. from the lighting circuit are led to a Thordarson T-6912 high-power transformer, *T*, from which two pairs of leads carry 5 amperes at 2.5 volts to tube filaments, *Y*. Two other pairs of leads carry, respectively, 3 amperes at 5 volts and 0.1 ampere at 400 volts to the type-80 rectifying tube. Two 30-henry chokes, *L*₁, and three 8-microfarad Sprague electrolytic condensers, *C*₁, bring the rectified current to a type-D Electrad Truvolt adjustable 75-watt 10,000-ohm resistor, *D*, from which the different voltages shown in the figure are tapped off. A mounted Bliley-type single-quartz crystal, *B*, ground to a frequency of 525 kilocycles is used in the fixed oscillator. The variable condenser, *C*₇, shown in this oscillator is of about 100 μmf . capacity, and is adjustable by means of a rod extending through the case, but is not altered once the oscillator functions properly.

The precision condenser, *C*_{*P*}, of the variable oscillator on which the actual capacity measurement is made may consist of a single instrument never adjusted above the total capacity value, about 1100 μmf . in this particular apparatus, necessary to give the matching frequency, or it may consist of a smaller and presumably more sensitive instrument in parallel with additional fixed condensers which may be switched in and out. A series condenser of capacity *C*_{*S*}, about 100 μmf ., may be thrown in series with the measuring cell of capacity *C*_{*x*} attached to mercury cups, one of which is soldered to the grounded shield. If *C*_{*x*} is too large to be measured on *C*_{*P*}, a lower total capacity, *C*, is obtained by switching to the series arrangement in which $1/C = 1/C_S + 1/C_x$. This series arrangement must, however, be employed with great care, as stray capacities and inductances may lead to serious errors.

With the quartz crystal rigidly mounted and housed in a Dewar tube and the variable oscillator thermostated, Stranathan reports that the apparatus has practically no frequency drift after the first hour required for warming up, while, if, in addition, all the air condensers are thermostated, the frequency drift is reported as not exceeding 1 or 2 cycles over the period of a day, irrespective of any reasonable changes in power-line potential or room temperature. This great stability is highly desirable should the apparatus be used for gases; but, as previously indicated, for the measurement of liquids, no thermostating of the quartz crystal, the oscillating circuits, or the condensers is needed. The precision of setting of the apparatus is far beyond the accuracy of calibration of the ordinary precision condensers. It has been applied by Stranathan³⁹ to gases. This

³⁹ J. Stranathan, *J. Chem. Phys.*, **6**, 395 (1938).

CONDENSERS

$C_3, C_5 = 140\text{-}\mu\text{f. two-gang variable.}$
 $C_1, C_4, C_{13}, C_{16}, C_{20} = 0.1\text{-}\mu\text{f. 600-volt paper.}$
 $C_6, C_7 = 0\text{-}30\text{-}\mu\text{f. air trimmer.}$
 $C_7 = 335\text{-}\mu\text{f. variable.}$
 $C_8 = 50\text{-}\mu\text{f. variable.}$
 $C_9 = 0.005\text{-}\mu\text{f. mica.}$
 $C_{10}, C_{17} = 0.01\text{-}\mu\text{f. 600-volt paper.}$
 $C_{11} = 8\text{-}\mu\text{f. 450-volt electrolytic.}$
 $C_{12} = 0.002\text{-}\mu\text{f. mica.}$
 $C_{14} = 0.25\text{-}\mu\text{f. 600-volt paper.}$
 $C_{15} = 4\text{-}\mu\text{f. 450-volt electrolytic.}$
 $C_{18} = 10\text{-}\mu\text{f. 25-volt electrolytic.}$
 $C_{19} = 300\text{-}\mu\text{f. mica.}$

RESISTORS

$R_1 = 20,000\text{-ohm 1-watt.}$
 $R_2, R_{10}, R_{17}, R_{19} = 0.5\text{-megohm } 1/4\text{-watt.}$
 $R_3, R_7, R_8, R_{16}, R_{20} = 50,000\text{-ohm 1-watt.}$
 $R_4 = 500\text{-ohm 1-watt.}$
 $R_5 = 7000\text{-ohm } 1/2\text{-watt.}$
 $R_6, R_{18} = 15,000\text{-ohm 1-watt.}$
 $R_9, R_{15} = 25,000\text{-ohm 1-watt.}$
 $R_{11} = 0.1\text{-megohm } 1/4\text{-watt.}$
 $R_{12} = 150\text{-ohm 1-watt.}$
 $R_{14} = 75,000\text{-ohm 1-watt.}$
 $R_{15} = 2000\text{-ohm 1-watt.}$
 $R_{21} = 0.5\text{-megohm potentiometer.}$
 $R_{22} = 1\text{-megohm } 1/2\text{-watt.}$
 $R_{23} = 5\text{ megohm } 1/4\text{-watt.}$
 $R_{24} = 30,000\text{-ohm 1-watt.}$

TUBES, ETC.

$V_1 = 6SJ7.$
 $V_2 = 6A8G.$
 $V_3 = 6SA7.$
 $V_4 = 6J5.$
 $V_5 = 6E5.$
 $X = \text{crystal.}$
 $M = 0\text{-}5\text{ ma. meter.}$
 $S_1, S_2 = \text{three-position two-gang switch.}$
 $J_1, J_2, J_3 = \text{phone jack.}$
 $P_1 = \text{socket for coaxial wire connector to dielectric cell.}$
 $P_2 = \text{socket for coaxial wire connector to external standard condenser.}$

COIL DATA

Frequency	Coil	Wire gauge, No.	Turns	Winding length	Coil diam. in.
500 kc.	A_1	29	106	Close wound	$1\frac{1}{2}$
	B_1	29	96	Close wound	$1\frac{1}{2}$
2.6 mc.	A_2	29	31	$7/8$ in.	$1\frac{3}{8}$
	B_2	29	28	1 in.	$1\frac{3}{8}$
5.0 mc.	A_3	22	22 $\frac{1}{2}$	Close wound	$1\frac{3}{8}$
	B_3	22	20	Close wound	$1\frac{3}{8}$

A_3 which may be changed to alter the operating frequency. The tuning of the oscillator is indicated by a 5-ma. milliammeter, M , in its plate circuit. With full capacitance in, the plate current is off the scale of the milliammeter; as the capacitance is reduced, a sharp dip in the plate current to about 1.5 ma. occurs as oscillation sets in. The capacitance is then further decreased until the plate current rises to 3 ma., at which point the oscillator is found to be more stable. The variable frequency oscillator employing a 6A8-type pentagrid converter tube, V_2 , is described as simple in construction and possessed of unusual stability. The output signals from the two oscillators are fed through two small trimming condensers, C_5 and C_6 , to the signal grids of a 6SA7 mixer tube, V_3 . The beat frequency signal produced is amplified by the 6J5 tube, V_4 , and fed into the grid circuit of the 6E5 indicator tube, V_5 , which acts as a sensitive grid-leak detector. With no signal on the grid of this indicator tube, its shadow angle is wide, but the angle decreases on receipt of a signal. The shadow is blurred when the beat frequency is high, but when the beat frequency falls below the persistence period of vision, a periodic blinking of the "eye" at the frequency of the beat note is seen. At zero beat the "eye" remains wide open. Low frequency beats down to one beat per second can be observed, since the two oscillators do not synchronize when their frequencies are brought close together as oscillating circuits sometimes do. The jack, J_1 , can be used for the injection of an audio signal from a tuning-fork oscillator used as in the first heterodyne beat method described. J_2 can be used for the attachment of a cathode-ray oscilloscope and J_3 for the attachment of an earphone. The earphone tells the observer whether he is tuning up or down and the simultaneous use of the tuning eye and the earphone gives the same performance as the cathode-ray oscilloscope. The dielectric cell is attached by a coaxial cable to the socket, P_1 , and the precision measuring condenser is similarly attached at the socket, P_2 . This apparatus is described as usable for any medium giving cell-leakage resistance greater than 10^4 ohms. With a frequency of 500,000 cycles, a setting of one beat per second gives an accuracy of 1 in 500,000, which, as in the case of the other heterodyne beat apparatus, is far greater than that of any condenser usable in measurements on liquids and solids.

4. Measurement of Gases

A heterodyne beat apparatus like one of those described above lends itself admirably to the measurement of gases. A precision must, however, be obtained in the gas measurements much greater than that in the liquid measurements, since the capacitance of a condenser filled with air at atmospheric pressure is less than 0.06% greater than that of the evacuated

condenser. Although polar gases with their higher dielectric constants exert much larger capacitance effects, it is desirable because of deviations from the ideal gas laws and, in some cases, because of low vapor pressures, to extend the measurements to the lowest possible pressures at which the capacitance changes can be accurately determined. The error in the dielectric constant value of the gas should not be more than one in the fifth place and should preferably be reduced to the sixth place. To obtain the necessary precision in the capacitance reading, the precision condenser used in measurements on liquids is placed in series with a small fixed condenser, or a condenser of large scale and capacitance of the order of 5 $\mu\text{f.}$ is used. The necessary circuit stability and reproducibility is obtained with the heterodyne beat apparatus by enclosing it in an air bath maintained a few degrees above room temperature and constant to about 0.2° by means of a small electric heater, a regulator, and a small electric fan, the motor of which is outside the bath or well shielded from the circuits. A metal or metal-covered box used for electric shielding may serve simultaneously as the air bath. The cell to be measured should be immersed in an oil bath kept constant to about 0.02° . A temperature variation ten times greater than this would not alter the dielectric constant of the gas by an amount equal to the usual experimental error, but the cylinders usually used in the cell condenser contract or expand appreciably with change of temperature. If the temperature change is the same for each cylinder, the capacitance of the cell changes so little that, only when the temperature change is of the order of 20° or more, is it necessary to make a correction for the condenser's dimensional changes. However, the electrical insulation between the cylinders provides such excellent thermal insulation that, in the cells commonly used, a small temperature change in the bath is communicated to the outer cylinder before it reaches the inner cylinder or cylinders. The resultant contraction or expansion of one cylinder while the other remains unchanged or lags behind may cause a change of capacitance equivalent to an error of several per cent in the value of $\epsilon - 1$ for the gas. Change of temperature within the condenser caused by change of pressure of the gas as it is admitted to or withdrawn from the cell may cause a similar change of capacitance, which necessitates a wait of half an hour or more for the condenser to attain uniform temperature. If a metal cell is used with the cell itself functioning as the outer cylinder of the condenser, the temperature lag is greatly reduced. It is necessary to shield the cell leads from the bath liquid to prevent large errors caused by small changes in the dielectric constant or in the height of the oil in the bath, changes which naturally accompany change in temperature.

If the substance to be measured is noncorrosive and gaseous at room temperature, it can be fed directly into the measuring cell through a system

containing a mercury manometer to determine its pressure. It may be stored under pressure in an ordinary steel gas cylinder or frozen with liquid air in a trap from which a desired quantity is allowed to evaporate into the cell or into which a quantity may be condensed from the cell. If the gas is corrosive, a capillary spiral, a Bourdon gage, or some other form of diaphragm gage may be used to determine the pressure. However, in the case of substances, corrosive or otherwise, which can be easily liquefied, a satisfactory device is a small glass manometric U tube⁴² placed close to the measuring cell. The glass tube connecting one end of the U tube to the measuring cell is wound with Nichrome wire, with which it is kept warm enough to prevent condensation of the liquid. At the beginning of an experiment, the liquid to be measured is introduced into a small trap or reservoir. It is frozen with dry ice or liquid air and the entire system is evacuated by means of a good oil pump or a mercury vapor pump. With the pumping system and mercury manometer cut off by a stopcock, the trap or reservoir is warmed and liquid is distilled over into the U tube until it is partially filled. A small quantity of well-dried air or nitrogen from a tank is admitted to the system until its pressure is sufficient to push the liquid in the U tube high enough in the arm toward the cell to reach the heated portion of the tube. A little of the liquid then evaporates into the cell and the pressure of the vapor pushes the liquid back down the arm of the U tube. The pressure of the vapor in the cell is given by the pressure of the nitrogen or air in the system, as read on a large mercury manometer, corrected by the small pressure difference indicated by the difference in heights of the liquid in the two arms of the U tube. This difference in height read on a millimeter scale is usually not more than 1 or 2 cm. and fluctuations of 1 or 2 mm. are unimportant since they correspond to less than 0.1 or 0.2 mm. of mercury. The pressure in the cell may be reduced by cooling the liquid in the U tube to condense vapor into it from the cell and removing small quantities of nitrogen or air from the gas system by pumping. With a little practice, the pressure may be controlled and observed with an accuracy determined mainly by the accuracy with which the mercury manometer is read.

The dielectric constant of a gas is dependent upon its density as determined by temperature and pressure. It is convenient to calculate a dielectric constant value at 760 mm. pressure and the absolute temperature T of the measurement from an easily derivable equation:

$$\epsilon - 1 = (760/C_0)(dC/dp)$$

where C_0 is the vacuum capacity of the measuring cell and dC/dp is the

⁴² C. T. Zahn, *Rev. Sci. Instruments*, 1, 299 (1930).

rate of change of the capacity of the cell condenser with change of the pressure of the gas in it. If the readings of the balancing precision condenser expressed in the same units as C_0 are plotted against the gas pressure in millimeters of mercury, the slope of the resulting curve gives the value of dC/dp from which the value of $\epsilon - 1$ may be calculated. Extension of the measurements to low pressures reduces or eliminates errors caused by deviations from the ideal gas laws, but reduction of the pressure below 30 or 40 mm. may, in many cells, cause excessive lag in the attainment of temperature equilibrium. The dielectric constant value thus calculated at 760 mm. is used to calculate the molar polarization, P , by means of the equation:

$$P = 22414T(\epsilon - 1)/273.1(\epsilon + 2)$$

The dipole moment value is best obtained by measuring P over a range of temperature so wide as to give an accurate direct determination of the constant b (see page 1617). However, as the result of errors in the dielectric constant measurements together with the frequent limitation of the temperature range by low vapor pressure and tendency toward thermal decomposition of the substance measured, it has become a common practice to calculate the moment from the total polarization as in the liquid measurements. When the same value of the moment is obtained at several different temperatures, assurance is given of the accuracy of the method.

Further details and variations of technique may be found in the original literature.^{29, 35, 43-50} A heterodyne beat apparatus⁵¹ of great precision employs a commercial crystal frequency standard, the General Radio Company 475-C frequency monitor, for the fixed oscillator, and the 661 frequency deviation meter of the same company for the beat frequency indicator. The discussion of this apparatus includes a treatment of inductance effects as a source of error in the capacitance measurements. The effects are very small in comparison to those which have been discussed in this chapter in connection with the higher frequency measurements with the Twin-T circuit, but, because of the high precision required in the gas measurements, may be appreciable sources of error if neglected.

⁴³ C. T. Zahn, *Phys. Rev.*, **24**, 400 (1924).

⁴⁴ H. A. Stuart, *Z. Physik*, **47**, 457 (1928).

⁴⁵ K. B. McAlpine and C. P. Smyth, *J. Am. Chem. Soc.*, **55**, 453 (1933).

⁴⁶ R. H. Wiswall and C. P. Smyth, *J. Chem. Phys.*, **9**, 352 (1941).

⁴⁷ E. C. Hurdis and C. P. Smyth, *J. Am. Chem. Soc.*, **64**, 2829 (1942).

⁴⁸ I. E. Coop and L. E. Sutton, *J. Chem. Soc.*, **1938**, 1269.

⁴⁹ H. E. Watson, *Proc. Roy. Soc. London*, **A143**, 558 (1934).

⁵⁰ H. E. Watson, G. G. Rao, and K. L. Ramaswamy, *Proc. Roy. Soc. London*, **A132**, 569 (1931).

⁵¹ J. G. Jelatis, Technical Report No. VII, Laboratory for Insulation Research, Massachusetts Institute of Technology, 1947.

5. Precision Condensers

Precision condensers have been constructed in which a plate of insulating material such as glass is moved forward or backward between the two parallel metal plates of the condenser, the capacity of which is a linear function of the position of the plate of insulator as read on a scale. In another type, the capacity is varied by moving one cylinder into another concentric cylinder, both cylinders being shielded by a third, which is large and usually grounded. The use of a worm drive and vernier scale makes both of these instruments, if properly constructed, capable of high precision. The precision condenser commonly used, resembling the ordinary variable radio condenser, consists of two sets of interlocking plates, the two sets carefully insulated one from the other, the one fixed and the other movable, its position read on a scale giving the capacity. Instruments (type 722 Precision Condenser, General Radio Company, Cambridge, Mass.) are obtainable in which the use of a worm drive makes possible a scale which can be set and read to one part in 25,000 of full scale. Maximum capacitances of 110, 500, 1000, and 1100 $\mu\text{f.}$ are available; smaller maximum values can be obtained by the removal of plates. The normal calibration curves supplied with the instruments are accurate to 1.0 $\mu\text{f.}$, but more precise curves with the so-called "worm correction" can be obtained to give an internal consistency of about 0.1 $\mu\text{f.}$ or 0.1%, whichever is larger. The investigator of dielectric constants will, however, find it more economical and probably more satisfactory to carry out his own calibration with the instrument in place in the apparatus to be used for the dielectric constant measurement.

Since the dielectric constant is obtained as the ratio between two capacity readings, the unit in which the capacity is expressed is immaterial. A convenient method of calibration is to measure a small fixed condenser of, perhaps, 10 $\mu\text{f.}$ capacity, the exact value of which does not need to be known, step by step over the entire portion of the precision condenser scale which is to be used in the dielectric constant measurements. A small dielectric constant cell may be used for the fixed condenser, or one may very easily be constructed. The dielectric constant apparatus is balanced with the precision condenser set at the maximum value at which it is to be used, while in parallel with it is a compensating condenser turned down close to its minimum value. The compensating condenser may best be a second precision condenser, if it happens to be available, because of its ease of precise adjustment; but, if such a condenser is not available, a cheap variable radio condenser with a little vernier condenser attached in parallel will serve. The small fixed condenser is then attached in parallel with the precision condenser to be calibrated, and the latter is turned down until the apparatus is balanced once more, the difference between the two con-

denser readings giving the capacity of the fixed condenser in scale divisions. The fixed condenser is then removed and the compensating condenser turned up until the apparatus is balanced. The fixed condenser is attached again and the precision condenser turned down until the apparatus is balanced again. This process is continued until the capacity of the small fixed condenser has been read in scale divisions in successive steps over the range of the precision condenser to be used.

A table is constructed in which the first column gives the precision condenser readings from the lowest to the highest value on the scale. The differences between the successive readings are averaged to obtain an average apparent capacity for the fixed condenser expressed in scale divisions. This average value is then added to the lowest condenser reading at the top of the first column to obtain a value differing by a very small amount from the second reading in the first column; and the difference is listed as a correction to the second reading opposite to it in a parallel column. The average apparent capacity of the fixed condenser is now added to the second reading in the first column to obtain a value differing slightly from the third reading. This difference is set down as a correction to the third reading; and the process is continued until the highest reading is reached, where the correction, having sometimes reached a value as high as 10 or 12 scale divisions, returns to zero. The corrections are now plotted as ordinates against the corresponding condenser readings in the first column as abscissae to obtain a curve from which the correction corresponding to any condenser reading between the highest and lowest value may be read off. The capacity of the dielectric constant cell is obtained in terms of these corrected scale divisions as the difference between two precision condenser readings to each of which is added or subtracted a correction read from the curve. The detailed form of the curve commonly has a zigzag variation of several tenths of a scale division caused by the action of the worm drive. It is a useful thing to check this curve carefully running both up and down the condenser scale, for not only is it desirable that the investigator should have an accurate calibration curve for the condenser but also the process of acquiring it gives him useful information as to the accuracy and reproducibility of his measurements. It should be fairly easy to reproduce a single setting on the condenser to 0.1 scale division, but uncertainties in the calibration curve are likely to be somewhat greater. The actual balance in the heterodyne beat apparatus which has been described is much more sensitive than the reading of the condenser.

For the measurement of gases, the necessary precision may be obtained by placing a small fixed condenser in series with one of the condensers previously described. A condenser of sufficiently small capacitance and large scale for direct measurements in parallel has been made⁴⁶ by the removal of

all but one plate on one side and two on the other of an old General Radio Company precision condenser. The condenser thus constructed from one with an original capacitance of 1500 $\mu\text{mf.}$ had a variable capacitance of 7 $\mu\text{mf.}$ with a scale of 2500 divisions. One constructed by Watson⁴⁹ on the principal of one cylinder screwing axially into another had a capacitance range of 1.2 $\mu\text{mf.}$ traversable in approximately 60 turns. An instrument of similar type used in the previously described precision apparatus manufactured by *Kipp & Sonen* had a capacitance of 0.001 $\mu\text{mf.}$ per scale division with a total capacitance of 5 $\mu\text{mf.}$

6. Dielectric Constant Cell

The condenser to contain the material to be measured has sometimes been constructed of parallel plates insulated from one another. Another type is similar to the ordinary variable radio condenser with two sets of interlocking plates, one fixed and the other adjustable. The capacity is determined for two settings of the condenser, for each of which the plates are immersed once in air and once in the material to be measured. If ΔC is the capacity difference between the two readings with air and ΔC_z , the difference with the plates immersed in the material, the dielectric constant, ϵ , of the latter equals $\Delta C_z/\Delta C$. This cell has the great advantage that it eliminates the lead and insulation capacity of the condenser from the determination and gives an absolute value for the dielectric constant. The disadvantages are complexity of construction, the difficulty of obtaining a convenient insulating material which is not attacked by the liquids measured, the considerable quantity of material normally required to cover the plates, and difficulty in adjustment at other than room temperature.

The simplest cell to construct and one that is quite satisfactory in use consists of concentric metal cylinders insulated from one another by small pieces of mica. The detailed construction of a three-cylinder condenser and containing vessel has been described elsewhere.^{35, 52} The capacity in micromicrofarads of a condenser consisting of two coaxial cylinders of length l is $0.2416 l/\log_{10} (r_1/r_2)$, where r_1 is the internal radius of the outer cylinder and r_2 , the external radius of the inner cylinder. This formula is used in determining the size of the cylinders to be used in the condenser. A separation of at least 0.5 mm. is desirable to facilitate the escape of possible gas bubbles from the space between the cylinders. A length of 5 to 8 cm. and a diameter of 1 to 2 cm. are convenient dimensions, while an air capacity of 50 to 200 $\mu\text{mf.}$ is suitable for most solution

⁴⁹ C. P. Smyth and S. O. Morgan, *J. Am. Chem. Soc.*, **50**, 1547 (1928).

measurements. Three concentric cylinders may be used advantageously, the middle one a few millimeters shorter than, and shielded by, the outer and inner cylinders, which are connected together and usually to ground. This three-cylinder condenser is equivalent to two parallel condensers, each with a capacity calculable by the formula for two coaxial cylinders. If the materials to be used do not attack brass, standard brass tubing of suitable diameter may be selected and sawed to the desired lengths. The brass may be protected by gold plating, but unless this plating is expertly done it may prove more economical to procure gold or platinum cylinders if the cell is to be used for more than a year. Of course, stainless steel, monel metal, silver, copper, etc., may be used in some cases. Glass tubes with a film of platinum or silver on the inside of the outer tube and on the outside of the inner tube may be used. The metal tubes are held apart by forcing small strips of mica 5 to 6 mm. long and 2 to 3 mm. wide between them at three points at the top and at the bottom. Glass and quartz are preferable to mica but are harder to adjust and more likely to break and slip out. Micalex and Teflon (polytetrafluoroethylene) also appear to be useful in cell insulation.

The cylinders are enclosed by a close fitting glass tube, to which a smaller tube leads from above for the admission of liquid, and from the bottom of which a smaller tube may curve upward to parallel the admission tube. Liquid may be expelled through this smaller tube by blowing through the admission tube. Two platinum lead wires fused or soldered to the condenser cylinders are brought up through glass tubes sealed to either side of the top of the vessel and terminating in small cups filled with mercury, into which brass rods are dipped to connect the condenser to the measuring apparatus. The two leads should be as widely separated as possible to reduce the capacity and inductance between them. The tubes containing them as well as the inlet and outlet tubes should extend about 20 cm. above the cell in order to permit deep immersion of the vessel in a constant-temperature bath. The depth of the immersion must always be the same in order that the lead capacity of the cell may be constant. The bath liquid should be one of low dielectric constant such as kerosene. The dielectric constant of water is so high that a change of height of a few millimeters caused by its evaporation from the bath will give rise to a considerable error.

The cell always has a certain fixed capacity, C_L , through the insulation of the plates or cylinders from one another and between the leads. Its capacity, when measured with air in it, the small deviation of the dielectric constant of air from 1 being neglected, is, therefore, $C_0 + C_L$, and, when measured with a liquid of dielectric constant ϵ in it, is $\epsilon C_0 + C_L$. By using a pure liquid of known dielectric constant, such as benzene ($\epsilon =$

2.276 at 25° C.), C_0 and C_L may be determined and used for the measurement of the dielectric constant of an unknown material.

It should be noted that the dielectric constant measuring apparatus is usually constructed so that one side of the condenser to be measured is grounded. It is, therefore, only the ungrounded side that needs to be connected or disconnected when the cell is attached to or removed from the measuring apparatus. This can be done by moving one end of a light brass rod which is just thick enough to be rigid and long enough to reach from the mercury cup of the cell to a mercury cup on the end of a fine rod supported by a small piece of bakelite or hard rubber screwed over a 1 to 2 cm. hole in the shield of the measuring apparatus. In precise measurements on liquids, it may be preferable to leave the cell in permanent position connected in parallel with the precision condenser, measuring the change in capacitance produced by filling and emptying the cell. The cell is then filled, emptied, and dried without removal from the thermostat. This procedure is always followed in the case of gas measurements.

Cells for the measurement of gases are similar in type to those used for liquids, but are usually of higher capacitance, 200 to 500 μf . being sufficient. Resistance of the metal to corrosion is even more important than in the case of cells for liquid measurements. The cell originally built by Zahn⁴³ of two concentric cylinders of gold-palladium alloy insulated from one another by quartz disks mounted in the ends of the cylinders has, with occasional rebuilding, given over twenty years of service. Stainless steel or heavily gold-plated brass or copper may be used. Rigidity of construction is more necessary in the gas cell than in that for liquids. An excellent type of cell is one in which the low-potential or grounded cylinders are welded or soldered with silver or gold to a disk which forms the bottom of the condenser, while the high-potential cylinder or cylinders similarly connected to a plate fit concentrically inside them. A high capacitance can thus be obtained, while the thermal lag is reduced to a minimum.

VI. CRITICAL SUMMARY

Dipole moment values are employed principally to determine the geometric structures of molecules and to investigate the nature of chemical bonds and the contributions of polar structures in resonance. The dielectric constant depending upon the presence of dipoles may sometimes be used as a means of analyzing mixtures. The dipole moment often does not distinguish uniquely among two or more geometric structures possible for a molecule, but may give strong evidence which, combined with other evidence, leads to a definite conclusion as to structure.

The determinations of dipole moment by a molecular beam method^{53, 54} and from measurements of dielectric absorption⁵⁵ have not been considered in this chapter, since, for most molecules, these methods are less accurate, more difficult, and often impossible. For the accurate determination of the dipole moment of a molecule free of environmental influence, measurement of the dielectric constant of the substance in the vapor state generally provides by far the best method, and, for this measurement on the vapor, the heterodyne beat method is the most convenient and dependable. Since, however, the great precision required in the gas measurement makes this method difficult, and since the low vapor pressures or thermal instability of many substances make the gas measurement impossible for them, the great majority of measurements are made upon solutions of the substances in nonpolar solvents. For such measurements, the heterodyne beat method, without the refinements and special precautions necessary with gases, is both convenient and accurate. A resonance circuit may also be employed and a fair degree of accuracy may be obtained with the very simple modified resonance circuit which has been described. In the unusual case that the liquids to be measured have any considerable conductance, it is necessary to use a bridge or a Twin-T circuit, which is also satisfactory for nonconducting liquids.

⁵³ R. G. J. Fraser, *Molecular Rays*. Cambridge Univ. Press, London, 1931, Chapter 6.

⁵⁴ H. K. Hughes, *Phys. Rev.*, **70**, 570 (1946).

⁵⁵ D. H. Whiffen and H. W. Thompson, *Trans. Faraday Soc.*, **A42**, 114 (1946).

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CONDUCTOMETRY

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I. INTRODUCTION

The transport of electricity through matter is effected by the movement of electric charges. These may be electrons, as in metallic conductors,

positive and negative ions in electrolytic conductors, or charged particles, as well as ions, in colloidal conductors. Most conducting solutions, fused salts and many solid ones are electrolytic conductors.

The extent to which electric current flows through an electrolytic solution depends on the applied voltage and on the number, charge, and mobility of the ions or other charged particles which are present. The number present depends on the concentration and, with weak electrolytes, on the extent of ionization, which also depends on concentration, on temperature, on pressure, and on the nature of the solvent. The mobility depends on the geometric nature, of the ions, which may be solvated, on the viscosity, and on the temperature.

Electric current can be led into and out of an electrolytic solution through solid electrodes, other electrolytic systems, or gaseous conductors. The cell as a whole, however, generally contains two metallic electrodes, an anode, and a cathode, at which chemical reactions take place. Negative ions, or anions, move towards the anode where oxidation occurs; positive ions, or cations, move towards the cathode where reduction occurs. The nature of these chemical changes, or electrode reactions, we shall not discuss in any detail in this chapter. They concern us only insofar as they may introduce difficulties into conductivity determinations.

II. ELECTROLYTIC CONDUCTANCE

1. Definitions and Units

According to Ohm's law the *current*, I , flowing in an electric conductor is directly proportional to the *electromotive force*, E , and inversely proportional to the *resistance*, R , in the conductor. The respective units, ampere, volt, and ohm, for these quantities are defined so that:

$$I = E/R \quad (1)$$

The *resistance*, $R = E/I$, of an homogeneous material under given conditions depends not only on the intrinsic property but also on the quantity and shape of the sample. If the material is of uniform cross section with an area of a cm.² and is l cm. in length, its longitudinal resistance is given by the equation:

$$R = \rho \cdot l/a \quad (2)$$

in which ρ is the intrinsic property, *specific resistance*, that is, the resistance per centimeter with a uniform cross-sectional area of one square centimeter. The reciprocal of resistance is termed *conductance*, and the reciprocal of *specific resistance*, ρ , is termed *specific conductance*, κ .

The specific conductance of an electrolytic solution depends only on the ions present, and therefore it varies with their concentration. To focus our attention on the ionic nature of the solution the quantity, *equivalent conductance*, Λ , is used. It refers to a particular electrolyte, and may be defined as the conductance, due to that electrolyte, of a layer of solution one centimeter long having a uniform cross section and containing one equivalent of this solute. It is related to the specific conductance, κ , by the equation:

$$\Lambda = 1000\kappa/C \quad (3)$$

in which C is the concentration in gram equivalents per liter of solution.

The analogous quantity, *molar conductance*, Λ_m , is given by:

$$\Lambda_m = 1000\kappa/C_m \quad (4)$$

where C_m is the concentration in moles per liter. The ratio of Λ_m to Λ is thus the number of positive (or negative) ionic charges obtainable from one molecule of the substance.

The equivalent conductance of an electrolyte, salt, acid, or base varies with concentration in a given solvent at a specific temperature and pressure. This may be due in part to a change in the number of conducting ions and in part to a change in the ionic mobilities.

The ionic mobility is defined as the net ionic velocity in the direction of the electric potential per unit field (one volt per centimeter). In aqueous solutions, as well as in many organic solvents, the equivalent conductance of electrolytes decreases with increasing concentration from the maximum values corresponding to infinite dilution. An explanation of this phenomenon involves a consideration of several theoretical points.

2. Theory of Electrolytic Conductance

A. GENERAL

Arrhenius proposed the classical ionic or electrolytic dissociation theory in 1883. He assumed that the decrease in equivalent conductance of an electrolyte with increasing concentration was due to a decrease in the proportion of conducting ions, but that their mobilities remained unaltered. Thus, he expressed the "degree of ionization," α , as the ratio of the equivalent conductance, Λ , at a given concentration, to the value extrapolated at infinite dilution, Λ_0 :

$$\alpha = \Lambda/\Lambda_0 \quad (5)$$

This theory appeared to hold fairly well for weak electrolytes such as acetic acid in water, but not for strong ones such as most salts, strong acids, and

bases. The difficulty lay in the assumption that the ionic mobilities are independent of concentration.

Milner¹ attempted a quantitative mathematical calculation for the inter-ionic, electrostatic force effects of the ions on themselves, but his theoretical treatment led to serious mathematical difficulties. It remained for Debye and Hückel² to formulate an interionic attraction theory in more useful form. A further improvement in the theory, as applied to electrical conductance, was presented by Onsager.³

In the interionic attraction theory of electrolytes, the properties of the solutions are considered to be due to the interplay of electrostatic forces and thermal motion. The first of these tends to give the ions a definite arrangement, and the second tends to produce a random distribution. With these ideas it is possible to account for varying ion mobilities, and also thermodynamic properties, with changing concentration. The conductance variations of dilute solutions of strong as well as weak electrolytes can thus be satisfactorily explained.

As a result of electrostatic (coulomb) forces and thermal motion, any selected ion in a solution, a positive one for instance, will, on a time average, have more negative ions near it than if the distribution were purely random. This is known as the "ion atmosphere" of the selected ion. From a thermodynamic point of view, the effect of the presence of the ion atmosphere is to reduce the activity coefficients of the ions. Its effects on electrolytic conductance are twofold, both tending to decrease the ionic mobilities with increasing ion concentration. They are the, so-called, "electrophoretic effect" and "time of relaxation effect."

When an electric potential is applied, the ion atmosphere, which has a net charge equal but opposite in sign to that of the central ion, tends to move with its associated solvent molecules in a direction opposite to that of this ion. This produces a slowing up of the central ion, which, in effect, moves against a countercurrent of solvent amounting to an increase of viscous drag (Fig. 1). Because of its analogy to the retarding effect of the solvent on the motion of colloidal particles in an electric field, this phenomenon has been called the *electrophoretic effect*.

When no external electric field is applied on a solution, the ion atmosphere is spherically symmetrical with its electrical center of gravity at the central ion. If, however, the ion is made to move by an external field, the ion atmosphere must also move to adjust for this change. This takes place rapidly (during the short "time of relaxation") but not instantaneously,

¹ S. R. Milner, *Phil. Mag.*, **23**, 551 (1912); **25**, 743 (1913).

² P. Debye and E. Hückel, *Physik. Z.*, **24**, 305 (1923).

³ L. Onsager, *Physik. Z.*, **27**, 388 (1926); **28**, 277 (1927).

so that there results a distortion of the ion atmosphere, whose electrical center of gravity is shifted to a point behind the central ion, producing an electrostatic retarding force which slows up its motion (Fig. 2). This phenomenon is called the *time of relaxation effect*.

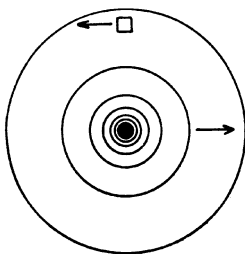


Fig. 1.—Electrophoretic effect.

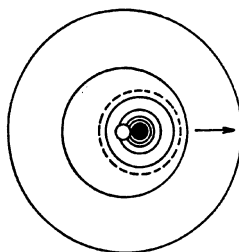


Fig. 2.—Time of relaxation effect.

In very dilute solutions the "thickness" of the ion atmosphere decreases with the square root of the ionic concentration and with ionic charge, or, more precisely, with the square root of the ionic strength. The retarding effect on ionic mobility due to both the electrophoretic and time of relaxation effects turns out to be inversely proportional to this thickness, which is usually of the order of angstroms. Moreover, the former is proportional to the ionic mobility at infinite dilution, and the latter to the fluidity of the solvent.

The quantitative conductance equation for very dilute electrolytic solutions derived by Onsager³ based on these considerations is:

$$\Lambda = \Lambda_0 - (\alpha\Lambda_0 + \beta)\sqrt{C} \quad (6)$$

where C is the ion concentration and the constants α and β depend on the nature of the solvent and the temperature. For uni-univalent electrolytes:

$$\alpha = \frac{8.20 \times 10^8}{(DT)^{1/2}} \text{ and } \beta = \frac{82.4}{(DT)^{1/2}\eta}$$

D being the dielectric constant at the absolute temperature, T , and η being the viscosity.

It has been shown that the Onsager equation is the correct limiting expression for the conductance of *strong* electrolytes.^{3,4} This is shown graphi-

⁴ D. A. MacInnes, *The Principles of Electrochemistry*. Reinhold, New York, 1939.

cally in figure 3 for the equivalent conductance of several salts in water at 25° C., in which the limiting slopes of the curves are quantitatively in excellent accord with those calculated from the theory. However, positive deviations from the theoretical (dotted) conductance slopes will be noted

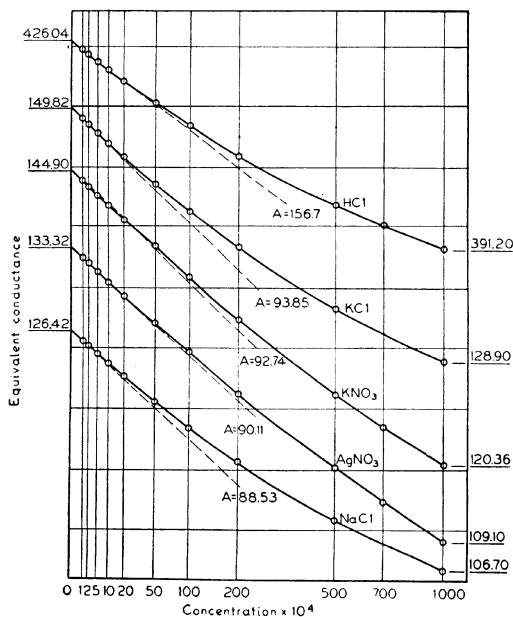


Fig. 3.—Equivalent conductance of typical uni-univalent electrolytes in water at 25° C.

as the concentration is increased. These can be accounted for by an empirical extension of the Onsager equation,⁵ namely:

$$\Lambda_0 = \Lambda'_0 - AC - BC \log C + DC^2 \quad (6a)$$

in which $\Lambda'_0 = (\Lambda + \beta \sqrt{C}) / (1 - \alpha \sqrt{C})$ from equation (6). In many cases, such as for most uni-univalent salts and strong acids in water up to a concentration of about 0.1 N, the last two terms in (6a) can be omitted.

⁵ T. Shedlovsky, *J. Am. Chem. Soc.*, **54**, 1405 (1932). T. Shedlovsky and A. S. Brown, *ibid.*, **56**, 1066 (1934).

If the Onsager equation had been derived for equivalent resistance instead of equivalent conductance it would be:

$$\frac{1}{\Lambda} = \frac{1}{\Lambda_0} + \left(\frac{\alpha \Lambda_0 + \beta}{\Lambda_0^2} \right) \sqrt{c} \quad (6b)$$

This equation is in close accord with measurements on strong electrolytes up to higher concentrations than equation (6),^{5a} but not to as high concentrations as equation (6a). However, as we shall point out later, it is very useful in analyzing conductance data on weak electrolytes.

B. EFFECT OF HIGH FIELD STRENGTH

It was observed by Wien⁶ that the equivalent conductance of electrolytes appeared to be increased at high potential gradients. This effect is in accord with the interionic attraction theory. When the velocity of ions due to high field strengths becomes sufficiently great, the ion atmospheres hardly have time to form, with the result that both the electrophoretic and time of relaxation effects become correspondingly diminished. In accordance with the Wien effect observations, the theory thus predicts an asymptotic approach of Λ vs. field strength curves to the Λ_0 values. Also, as would be expected, the relative increase in conductance is most marked in the cases of relatively concentrated solution of high valence electrolytes in which the interionic effects are most marked.

An unexpected experimental result was the large Wien effect shown by weak acids and bases. It appears, therefore, that for such weak electrolytes high field strengths tend to produce increased ionization.

C. EFFECT OF HIGH FREQUENCY

In order to avoid complications arising from polarizing effects at the electrodes, it is customary to carry out conductance measurements with alternating current. It was shown by Debye and Falkenhagen⁷ that when sufficiently high frequencies of current (over 5 megacycles) are used the equivalent conductance is increased, approaching a limiting value somewhat lower than Λ_0 . The theoretical explanation of this phenomenon lies in the fact that at high frequencies the ion atmosphere does not fail to form, as in the Wien effect, but has insufficient time to distort fully. Consequently, the time of relaxation effect tends to vanish, although the electrophoretic effect retains its full influence. Thus, as frequency is in-

^{5a} T. Shedlovsky, *J. Franklin Inst.*, **225**, (1938).

⁶ M. Wien, *Ann. Physik*, **83**, 327 (1927); **85**, 795 (1928); **1**, 400 (1929).

⁷ P. Debye and H. Falkenhagen, *Physik. Z.*, **29**, 121, 401 (1928). O. M. Arnold and J. W. Williams, *J. Am. Chem. Soc.*, **58**, 2613, 2616 (1936).

creased, equivalent conductance approaches not Λ_0 but $\Lambda_0 - \beta\sqrt{C}$ (see Eq. 6).

III. MEASUREMENT OF ELECTROLYTIC CONDUCTANCE

1. Kohlrausch Method

The passage of current from an electrode to a solution causes a chemical reaction which may produce changes in the composition of the solution in that region. Moreover, potentials at the electrodes may also thus arise, with the introduction of serious errors in the measurements, unless such "polarization" effects can be made negligible.

To overcome such difficulties, alternating currents are generally used in making conductance measurements so that polarization is constantly reversed. Platinum electrodes with a light coating of "platinum black," deposited electrolytically, are used. The "platinization" (platinum black which is an adherent coating of finely divided platinum) greatly increase

the surface and has a catalytic effect materially reducing polarization. The measurement of resistance, from which the conductance is obtained, is made with the help of a Wheatstone bridge. This method was first used by Kohlrausch (1875) and is still almost universally employed.

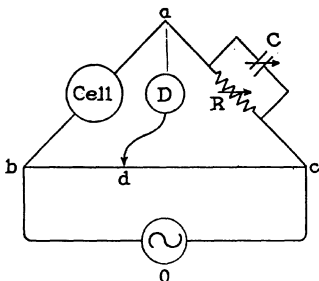


Fig. 4.—Kohlrausch's bridge circuit.

A schematic arrangement of the Kohlrausch equipment is shown in figure 4. The conductivity cell forms one arm of a Wheatstone bridge, ab , a standard variable resistance box, R , forms another arm, ac , and a calibrated slide wire resistance, bc , provides the third and fourth arms, bd and cd . Alternating current is supplied to the bridge by some source of a. c. power, O , across bc , and a detector telephone, D , is connected across ad . Since, with alternating current, reactances, *i. e.*, capacities and inductances, as well as resistances are of importance in obtaining balance in the bridge, a variable condenser, C , is provided for the purpose. When the position of contact d is adjusted for zero or minimum sound in telephone detector D , the potentials of points a and d are equal, or very nearly so. Then the following condition obtains:

$$R_{\text{cell}}/bd = R/dc \quad (7)$$

from which the resistance of the cell, R_{cell} , can be computed since the three other factors are known.

When the bridge is accurately balanced, the reactances, X , as well as the resistances must be adjusted, so that:

$$R_{ab}/R_{bd} = R_{ac}/R_{dc} \quad (8)$$

and:
$$X_{ab}/X_{bd} = X_{ac}/X_{dc} \quad (9)$$

In most recent work, the slide wire has been abandoned and fixed ratio arms have been substituted, usually consisting of two closely adjusted, non-reactive resistances, that is, resistances with low distributed capacity and negligible inductance. The source of a. c. power is usually some form of vacuum-tube oscillator, and a vacuum-tube amplifier is used in conjunction with a telephone to obtain greater sensitivity in the measurements.

ALTERNATING CURRENT BRIDGE

The theory and design of alternating current bridges for measuring electrolytic solutions have been discussed by Jones and Josephs⁸ and by Shedlovsky.⁹ When a direct current flows through a conductor, leakage can take place only through inadequate insulation. With alternating currents the case is more complicated because the current may also leak away through electrostatic and electromagnetic linkings, commonly known as "stray couplings." Such stray couplings may be electrostatic capacities between the parts of the circuit and capacities between these parts and the ground. There may also be mutual inductive effects due to linkages with stray magnetic fields. The effects produced by these couplings vary with the frequency, being more troublesome at higher frequencies.

Stray electromagnetic couplings are particularly important in the case of parts of circuits, such as transformer coils, which have appreciable inductance. These mutual inductive couplings may be reduced by using materials of high permeability in the cores of the transformers, by shielding stray fields with screens of high permeability, or by sufficiently separating the branches of the circuit having high inductances from each other. In the case of a Wheatstone bridge for measuring electrolytic conductance, the only branches in the network capable of very serious inductive coupling with each other are the detector and the oscillator. The best plan is to avoid the necessity of electromagnetic screening, which is always difficult and seldom completely satisfactory. This is readily accomplished by removing the oscillator to a sufficient distance from the detector, which is more conveniently located near the bridge, and avoiding as much as possible the introduction of stray inductance in the bridge itself. A distance of 10 to 15 feet between the detector and the oscillator is usually sufficient,

⁸ G. Jones and R. C. Josephs, *J. Am. Chem. Soc.*, **50**, 1049 (1928).

⁹ T. Shedlovsky, *J. Am. Chem. Soc.*, **52**, 1793 (1930).

if troublesome inductive couplings exist. However, by using a resistance-coupled amplifier with "crystal" phones, it is possible to reduce this distance materially, so that the entire bridge with oscillator and detector can be assembled as a single, relatively compact unit.

Electrostatic couplings existing between various branches of a circuit, directly or to ground, cannot be eliminated entirely in this simple manner without introducing another difficulty. That is because excessive separation of all branches of the bridge from each other and from ground, besides being inconvenient, introduces additional inductance into the network due to the increased length of leads required. The result of this increased loop-

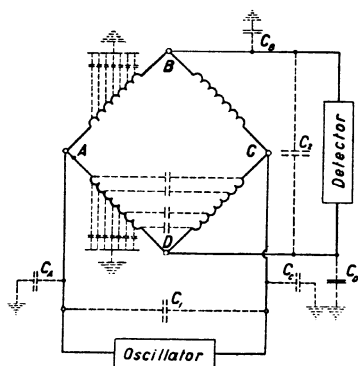


Fig. 5.—Capacity couplings in an alternating current bridge.*

ing is that, although capacitive couplings are reduced, inductances capable of disturbing the bridge balance are thus added, and the bridge balance may be changed by the inductive effect of the varying stray fields enclosed.

A bridge supplied with alternating current, indicating the position of some of the capacitive couplings, is shown in figure 5. The capacity paths from one lead of the oscillator to the other, either directly (C_1) or through ground (C_A and C_C in series), merely shunt the current input, and do not affect the bridge balance. Similarly, the coupling between the leads to the detector does not influence the bridge reading, merely shunting the detector. However, couplings are possible which may disturb the balance of the bridge. For example, current leaking from branch AD , through CB to the terminal B , and back to the oscillator through branch BC , would have an effect on the bridge balance. (It should be pointed out that bridge terminals A , B , C , and D include the leads appended to them since, if they are not too long, their resistance is usually negligible.) Although some of these numerous possible paths will tend to neutralize

each other, the effects of these paths must be controlled; otherwise, the bridge readings will depend on the relative positions of the units in the circuit and on the surroundings.

Precise measurements are not possible unless these troublesome couplings are neutralized by being made symmetrical with respect to the terminals of the detector. It is not possible to avoid electrical asymmetry entirely in the construction of the apparatus, particularly if the bridge is used for measurements with various frequencies of current, requiring readjustments in the oscillator, or perhaps different oscillators. Neither is it practicable to reduce these couplings so as to make them negligible

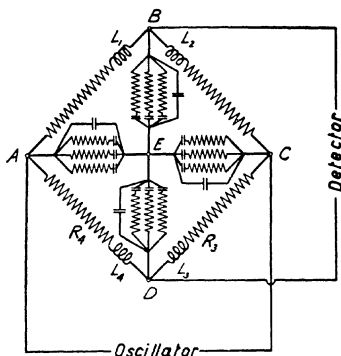


Fig. 6.—Effect of capacity couplings in an alternating current bridge.⁹

especially at the higher frequencies, and in the measurement of dilute solutions having large resistances. For these reasons it is necessary to provide means for controlling and balancing these disturbing effects. This is accomplished by the "Wagner earthing device."

It can be shown⁹ that the influence of stray capacity currents to a shield from a pure resistance makes it behave as if it were a somewhat larger resistance with a small appended inductance. Also, an effect appears amounting to the presence of impedances, such as resistance capacity combinations, from the ends of the resistance to the shield. Since three conductors join at each terminal of the bridge, this state of affairs is shown diagrammatically in figure 6, in which it is assumed that all the leakages take place to a common point, *E*, which is usually "earthed," and that interbranch leakages are negligible. It is at once apparent from this figure that paths *AEB* and *CEB*, for instance, act as shunts on arms *AB* and *BC*, respectively. Therefore, unless these paths happen to be adjusted in the same proportion as *AB* and *BC*, errors will result.

The Wagner earthing device eliminates the difficulty by providing adjustable impedances (resistance and capacity) across AE and CE . The procedure is first to balance the bridge itself with the detector across BD , then to balance the Wagner arms with the detector across BE or DE , and finally to readjust the main bridge balance with the detector in the original position. Thus, points B , D , and E are all brought to the same potential and no shunting errors can result.

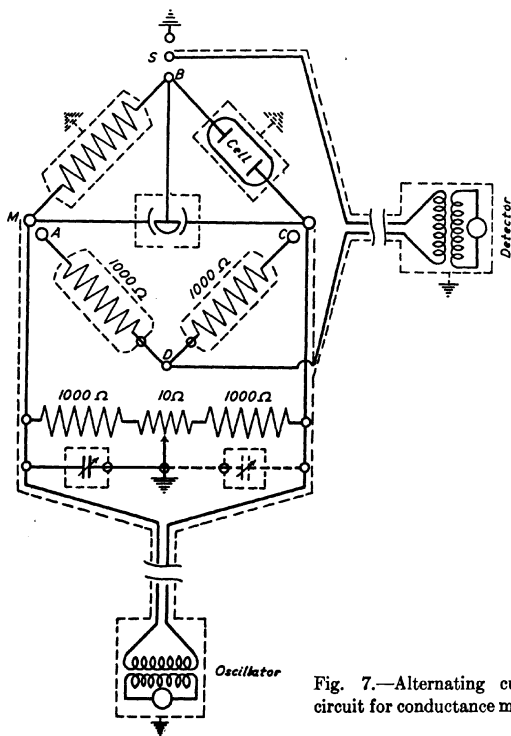


Fig. 7.—Alternating current bridge circuit for conductance measurements.⁹

Figure 7 shows diagrammatically a Wheatstone bridge circuit⁹ which has been used by the author for many years. The bridge is direct reading, that is, the ratio arms AD and CD are equal, so that, when both resistance and condenser balances are achieved for the main bridge and Wagner earth circuits, the resistance reading of box MB is equal to the resistance of the conductivity cell.

A simple schematic circuit for a vacuum-tube oscillator is shown in figure 8a. L_1 and L_2 are two fixed inductances and C is a condenser whose capacity may be varied if different frequencies of current are desired. The alternating current is carried to the bridge from inductance L_3 which picks it up electromagnetically from L_2 . The frequency usually employed is between 1000 and 3000 cycles per second. The circuit for the oscillator shown

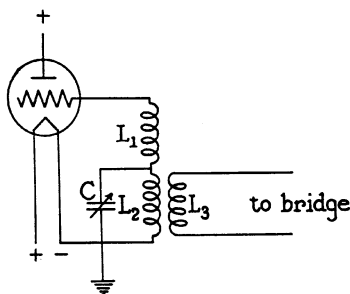


Fig. 8a.—Simple vacuum-tube audio oscillator.

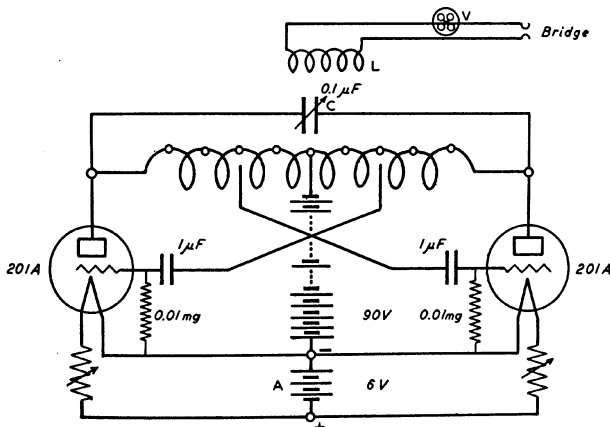


Fig. 8b.—Balanced vacuum-tube audio oscillator.⁹

$$T_1 - T_0 = T_0 - T'_1 = 50 \text{ mh.}; L, 100 \text{ turns.}$$

in figure 8b has been used by the author in conjunction with the bridge described above. V , in this figure, refers to a thermocouple ammeter which permits the measurement of the current supplied to the bridge.

A simple one-stage amplifier for use as the bridge detector is shown schematically in figure 9. It may

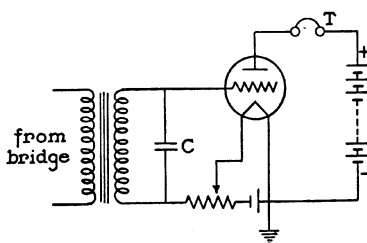


Fig. 9.—Simple vacuum-tube amplifier.

be coupled to the bridge through a transformer, as shown. Condenser C serves to "tune" the circuit to the frequency of current used in the bridge, and T represents the telephone. But, as mentioned above, if greater compactness of assembly is desired, the transformer is replaced by a resistance which couples the amplifier to the bridge through a condenser.

2. Direct-Current Method

Electrolytic conductance has sometimes been measured with direct current and nonpolarizable electrodes,¹⁰ such as silver-silver chloride electrodes in chloride solutions, mercury-mercurous sulfate in sulfate solutions, and hydrogen electrodes in acids. The current may be introduced into the cell, whose design is not critical through these electrodes and the resistance measured on an ordinary d. c. Wheatstone bridge. Another alternative is to introduce the current through two electrodes whose nature is not important as long as the electrode reaction products are not allowed to enter the portion of the cell containing the measuring, reversible electrodes. The potential difference between these latter ones is measured, as well as the current flowing through the cell, and the resistance between them is computed with the aid of Ohm's law. However, the direct-current method suffers from the limitation that it is applicable only to those electrolytes for which nonpolarizable electrodes can be obtained.

3. Conductivity Cells

For measuring electrolytic conductance, cells are used which are constructed of highly insoluble glass, such as Jena 16III or Pyrex, and firmly sealed platinum electrodes. For work of very high precision with extremely dilute aqueous solutions, quartz cells have been used. The resistance, R , of the solution in the cell is measured, as has been described above, and the conductance, κ , is computed from the equation:

$$\kappa = \frac{\text{cell constant}}{R} \quad (10)$$

If the cell is a tube of uniform cross section with the electrodes at the ends, the cell constant can be determined from geometric measurements, that is, length (cm.)/area (cm.²) (see Eq. 2). However, in practice, the determination of the cell constant is made by equation (10) after measuring the resistance of a solution having a known specific conductance. For this purpose solutions of potassium chloride are usually employed (Table I),

¹⁰ E. D. Eastman, *J. Am. Chem. Soc.*, **42**, 1648 (1920). L. V. Andrews and W. E. Martin, *ibid.*, **60**, 871 (1938).

TABLE I
SPECIFIC CONDUCTANCE, κ , OF POTASSIUM CHLORIDE SOLUTIONS
FOR CELL CONSTANT DETERMINATIONS^a

Temp., ° C.	Grams KCl per 1000 grams water		
	76.627	7.4789	0.74625
0	0.065176	0.0071379	0.00077364
18	0.097838	0.0111667	0.00122052
25	0.111342	0.0128560	0.00140877

^a G. Jones and B. C. Bradshaw, *J. Am. Chem. Soc.*, **55**, 1780 (1933).

since the specific conductance of several concentrations of this salt at several temperatures has been determined with a very high degree of accuracy.¹¹ The values given in this table do not include the conductance due to the water, which must be added and should be less than $\kappa_{\text{H}_2\text{O}} = 10^{-6}$ in work with dilute solutions.

It is good practice to choose cells with appropriate cell constants, so that the resistance will not fall far below 1000 ohms nor above 10,000 to 30,000 ohms. In the former case one avoids excessive polarization difficulties due to too high current densities; in the latter case one avoids errors due to insulation leakage. If, however, very high resistances must be measured, it is advisable to shunt the cell with a calibrated high resistance, 10,000 ohms, for instance, and then compute the cell resistance from the parallel circuit equation:

$$\frac{1}{R_b} = \frac{1}{R_c} + \frac{1}{R_s} \quad (11)$$

where R_b is the resistance of the parallel combination measured in the bridge and R_s is the resistance of the shunt.

Jones and Bollinger¹² have shown that many cells which have been commonly used in careful conductivity work suffer from a source of error inherent in the design. In such cells, in which the filling tubes are relatively close to the electrode leads, disturbing parasitic currents can flow through capacity-resistance paths, and these can produce apparent variations in cell constants. A variation of cell constant at high resistances had been observed by Parker,¹³ who attributed the effect to adsorption. Jones and Bollinger have, however, definitely proved that this so-called "Parker

¹¹ G. Jones and B. C. Bradshaw, *J. Am. Chem. Soc.*, **55**, 1780 (1933). G. Jones and L. T. Prendergast, *J. Am. Chem. Soc.*, **59**, 731 (1937). R. W. Brenner and T. G. Thompson, *J. Am. Chem. Soc.*, **59**, 2372 (1937). C. W. Davies, *J. Chem. Soc.*, **1937**, 432, 1326.

¹² G. Jones and G. M. Bollinger, *J. Am. Chem. Soc.*, **53**, 411 (1931).

¹³ H. C. Parker, *J. Am. Chem. Soc.*, **45**, 1366, 2017 (1923).

effect" disappears when the filling tubes and electrode leads in the cells are spaced in a manner which avoids appreciable stray currents. A properly designed cell is shown in figure 10. Contact is made to the sealed platinum electrodes through the mercury pools, shown in black.

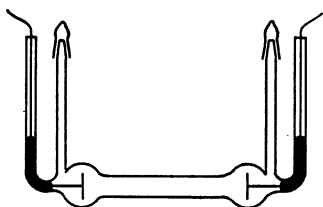


Fig. 10.—Conductivity cell (after Jones and Bollinger).

With very dilute solutions it is desirable to use a cell of relatively large volume, so that increasing concentrations can be built up and successively measured without risk of contamination from atmospheric or other impurities. Some workers have used cells consisting essentially of a flask with a pair of dipping electrodes, as is represented diagrammatically in figure 11a. However, dipping electrodes may also lead

to errors in conductivity measurements if high precision is important. The following brief analysis will make clear the reason for such errors and the direction of their effect on the measurements.

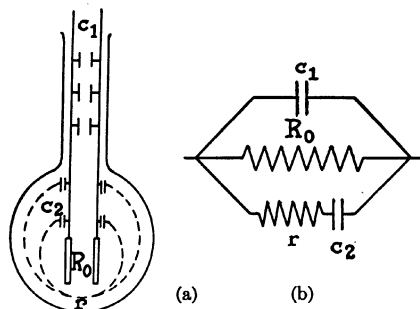


Fig. 11.—"Parasitic" currents in cell with dipping electrodes.

With the cell indicated in figure 11a, the total current measured in the bridge consists, when polarization is negligible, not only of the current flowing directly between the electrodes through the solution and a capacity current between the electrodes (including the leads), but also of a parasitic current which flows from the electrode leads by electrostatic capacity to the solution and then through it. This latter current is neither constant nor directly proportional to the resistance of the solution, and thus tends to produce apparent variations in the cell constant.

The electrical circuit for the cell in figure 11a is shown in sufficient de-

tail in figure 11b. Since this circuit is balanced in the bridge by a simple resistance and capacity in parallel, it can be shown theoretically that when the bridge is balanced:

$$\frac{1}{R_s} = \frac{1}{R_0} + \frac{1}{r} - \frac{1}{\omega^2 C_2^2 r^2 R_0^2} \quad (12)$$

in which ω is the angular frequency, $2\pi f$. The last term depends both on the frequency of current used in the bridge and on the resistance of the solution. To avoid this type of error, the electrode leads should be removed from within the flask, as has been done in the cell shown in figure 12.

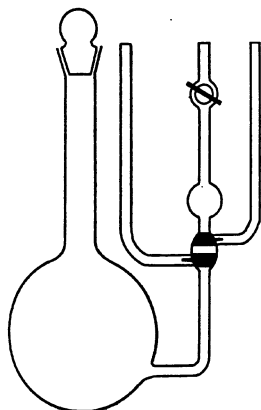


Fig. 12.—Flask-type conductance cell (Shedlovsky).

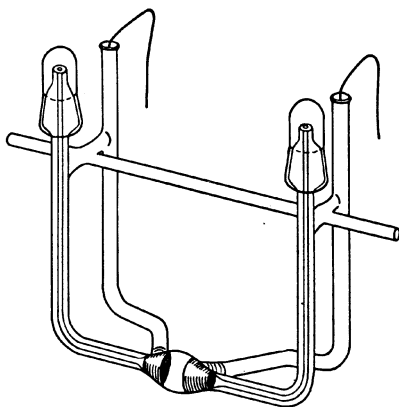


Fig. 13.—Conductance cell for protein and other solutions.

Here, the electrodes consist of truncated cones of platinum foil the outer surfaces of which are sealed to the glass.

This type of electrode construction is useful, not only because it assures the rigidity of the position of the electrodes, but also because it avoids the trapping of air bubbles in the cell, which should, of course, be completely filled with solution. The cell shown in figure 13 is particularly useful for this reason in work with protein solutions, which have a tendency to froth.

The "platinizing" of electrodes is carried out electrolytically after filling the cell, which is first cleaned with chromic sulfuric acid mixture or *aqua regia*, with a solution containing about 3% chloroplatinic acid and a trace (about 0.02%) of lead acetate. The lead tends to favor the formation of

a fine, adherent deposit. A current of several milliamperes is sufficient, and it is best to stop the electrolysis when the color of the electrode changes from a light brown to a black. The current is then reversed and the procedure repeated for the second electrode. The cell is then thoroughly washed with good distilled water, with which it should always be filled when stored.

In cases in which the substances to be investigated tend to be decomposed by platinum black, the electrodes may first be "platinized" and washed, then heated to redness until the deposit assumes a gray appearance on cooling. Smooth electrodes may be used, but definite polarization effects will occur, and it is then necessary to make measurements at several frequencies and to extrapolate the values to infinite frequency. A plot of resistance against the reciprocal of the square of the frequency is useful for the purpose, the extrapolation being carried to $1/f^2 = 0$.

It is obvious that the resistance of the leads connecting the cell to the bridge should be negligible or of known magnitude. If long leads are used, they should be enclosed in a metallic sheath which is grounded. Such shielded wire is commercially available.

To minimize temperature variations due to heat exchange between the electrodes of the thermostatted cell and the leads, it is convenient to immerse two test tubes containing mercury in the thermostat. Short copper wires provide contact between the electrodes and the mercury pools from which leads are then carried to the bridge.

4. Temperature Control

Accurate temperature control is very important in conductance measurements of precision because the temperature coefficient of conductance is about 2% per degree for most ions. The thermostat may have any design, but it is best to use oil rather than water in the bath. This is because the relatively high dielectric constant and conductance of water, as compared with mineral oil, results in sufficient leakage of electrical energy from the cell to introduce errors in some cases.

The degree of temperature regulation required will depend on the accuracy desired in the conductance determinations. For some purposes, as in conductivity titrations, it need not be very precise, and in this case many of the sources of error discussed above are also correspondingly less important. For other purposes, as in the accurate determination of ionization constants, or of concentrations, the control of temperature should be correspondingly greater. If an accuracy of one or two hundredths of a per cent is desired, the thermostat should regulate within a hundredth of a degree.

5. Conductivity Water and Solvent Correction

Distilled water always contains some carbon dioxide, sometimes ammonia and other traces of impurity. The conductance due to these substances is most serious in work with dilute solutions, which have a low conductivity.

For most general purposes, adequately pure "conductivity" water can be prepared by redistilling good distilled water to which a small quantity of alkaline permanganate or Nessler's solution has been added for the removal of ammonia. Precautions should be taken to avoid the carrying over of any spray, and a quartz, resistance glass, or pure tin condenser should be used. A considerable portion of the distillate is rejected, about one-fourth, and the rest collected in a thoroughly clean, steamed container with provision for avoiding contamination by carbonic acid or ammonia in the air. Such water should have a conductivity of less than 1.0×10^{-6} . For still purer water, special distilling equipment must be used.¹⁴

If organic solvents are used they should be not only carefully purified by distillation, but protected from contamination by moisture, which in many instances has a marked influence on the conductance.

The solvent conductance is determined by measuring its resistance in a cell of low cell constant, with a shunt, if necessary, as has been discussed above. The cell is refilled with fresh portions of solvent until a reproducible value is obtained. This procedure is also followed in all conductance determinations, to assure that no contamination occurs from material retained on the electrodes or glass walls from solutions previously present in the cell.

In general, the conductance of the solvent can be subtracted from the apparent conductance of the solution. This is true of most salt solutions. However, in certain cases the solvent correction is a complicated matter, depending on the substance being measured and on the nature of the impurity. Of course, the concentration of the solution is an important factor, since it determines the magnitude of the conductance and therefore the relative importance of the much smaller part played by the solvent. Thus, for example, in very dilute acetic acid solutions, the conductance due to a trace of carbonic acid in the original solvent is repressed, but not that due to salt impurities. In acetic acid solutions of higher concentrations, the exact form of the solvent correction becomes trivial because of its inconsequential magnitude in comparison with the total conductance.

¹⁴ C. A. Kraus and W. B. Dexter, *J. Am. Chem. Soc.*, **44**, 2468 (1922). I. Bencowitz and H. T. Hotchkiss, Jr., *J. Phys. Chem.*, **29**, 705 (1925). A. I. Vogel and G. H. Jeffery, *J. Chem. Soc.*, 1931, 1201.

6. Examples of Applications

A. WEAK ELECTROLYTES. IONIZATION CONSTANTS

In solvents of high dielectric constant, such as water, strong electrolytes appear to be completely ionized. On the other hand, in solvents of low dielectric constant no strong electrolytes exist.

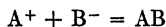
It is generally assumed that only a portion of a weak electrolyte is in the form of free ions while the rest is undissociated, and that an equilibrium exists between the two forms of the solute in accordance with the law of mass action. However, the undissociated solute need not consist of stable molecules bound by chemical or quantized forces. They may be ion pairs, as suggested by Bjerrum.¹⁵ The electrostatic force between a pair of ions of opposite charge which have approached each other closely, tends to hold them together as a dipole. Now, if the average kinetic energy of the solvent molecule is greater than the potential energy of the ion pair, it will soon be disrupted. This is probably the case for strong electrolytes in solvents with high dielectric constants. If, on the other hand, the average kinetic energy of the solvent molecules is less than the mutual potential energy of the ion pair, it will exist until it is struck by an exceptionally fast solvent molecule. This viewpoint predicts that there should be relatively more ion pairs when the dielectric constant is low, when the ions are small, when the charges are large, and when the temperature is low.

According to the Arrhenius dissociation theory, weak electrolytes were found to follow the Ostwald dilution law:

$$\Lambda = \Lambda_0 - \frac{c\Lambda^2}{K\Lambda_0} \quad (13)$$

an expression derived from the mass action law and the assumptions (a) that the ions are perfect solutes, and (b) that the ratio Λ/Λ_0 represents the degree of dissociation. However, the first assumption is true only as a very rough approximation in dilute solutions; and the second assumption can be valid only if the mobilities of the ions do not change with the concentration, which is contrary to fact.

The ionic equilibrium in a solution of a simple weak electrolyte is represented by the equation:



According to the law of mass action:

$$K = \frac{(A^+)(B^-)}{(AB)} = \frac{c^2 x^2 f^2}{c(1-x)f_u} \quad (14)$$

¹⁵ N. Bjerrum, *Danske Vidensk. Selskab.*, 7, No. 9 (1926).

in which K is the thermodynamic ionization constant, the parentheses refer to activities, c is the concentration, x is the degree of ionization, and f and f_u are the activity coefficients of the ions and "undissociated molecules" or ion pairs, respectively.

In dilute solutions, the ion pairs may be considered as normal solutes with $J_u = 1$. The activity coefficient, f , for the "free" ions can be computed from the Debye-Hückel equation:

$$-\log f = a\sqrt{cx} \quad (15)$$

in which cx is the ion concentration and the constant $a = 0.509$ in water at 25° C. for univalent electrolytes. It increases linearly with decreasing dielectric constant and absolute temperature.

The degree of ionization, x , can be obtained from the ratio of the actual equivalent conductance, Λ , to the equivalent conductance, Λ_e , of the completely ionized solute at the same ion concentration, that is:

$$x = \Lambda/\Lambda_e \quad (16)$$

If, for example, the weak electrolyte is an acid, HA, the values of Λ_e at different ion concentrations can be computed from three sets of conductance data on strong electrolytes.¹⁶ For example:

$$\Lambda_e = \Lambda_{H^+Cl^-} + \Lambda_{Na^+A^-} - \Lambda_{Na^+Cl^-} = \Lambda_{0(HA)} - A\sqrt{cx} + Bcx + \dots \quad (17)$$

$$x = \frac{\Lambda}{\Lambda_{0(HA)} - A\sqrt{cx} + Bcx + \dots} \quad (18)$$

The details of the computations for x involve a short series of approximations, since the ion concentration contains x as a factor.

If the ion concentration is not too high, the degree of ionization can be obtained from the conductance data on the weak electrolyte itself,¹⁷⁻¹⁹ using a modified form of the Onsager equation.^{5a} Thus:

$$x = \frac{\Lambda}{\Lambda_0} F \quad (19)$$

in which:

$$F = \left[\frac{Z}{2} + \sqrt{1 + \left(\frac{Z}{2} \right)^2} \right]^2$$

¹⁶ C. W. Davies, *J. Phys. Chem.*, **29**, 977 (1925). M. Sherrill and A. A. Noyes, *J. Am. Chem. Soc.*, **48**, 1861 (1926). D. A. MacInnes, *ibid.*, **48**, 2068 (1926). D. A. MacInnes, *The Principles of Electrochemistry*, Reinhold, New York, 1939.

¹⁷ R. M. Fuoss and C. A. Kraus, *J. Am. Chem. Soc.*, **55**, 476 (1933).

¹⁸ R. M. Fuoss, *J. Am. Chem. Soc.*, **57**, 488 (1935).

¹⁹ T. Shedlovsky, *J. Franklin Inst.*, **225**, 739 (1938).

or, approximately, $F = 1 + Z$. The function Z is defined as:

$$Z = \left(\frac{\alpha\Lambda_0 + \beta}{\Lambda_0^{1/2}} \right) \sqrt{C\Lambda}$$

Combining this expression for x with the mass action law there results:

$$\Lambda F = \Lambda_0 - \frac{F^2 f^2 \Lambda^2 C}{K \Lambda_0} \quad (20)$$

analogous to the older Ostwald dilution equation, or:

$$\frac{1}{\Lambda F} = \frac{1}{\Lambda_0} + \frac{f^2 F \Lambda C}{K \Lambda_0^2} \quad (21)$$

similarly analogous to an equation which had been used by Kraus and Bray.

The activity coefficient, f , can be computed from the Debye-Hückel equation:

$$-\log f = a\sqrt{cx} = a\sqrt{C\Lambda F/\Lambda_0}$$

The computations are first carried through with a provisional value of Λ_0 and plots are prepared of equation (21) or (20). These should be linear and extrapolated to a more nearly correct value of Λ_0 . As a rule, two or three series of computations and plots will yield a constant value of Λ_0 . The value of the ionization constant, K , can thus be found readily from the slope of the last plot.

B. CONCENTRATION AND SOLUBILITY

Conductance is a sufficiently sensitive function of concentration to be useful in its quantitative determination. Since the temperature coefficient of conductance is appreciable, about 2% per degree for most ions in water, good temperature regulation is essential for accurate work.

The procedure is obvious. A calibration conductance curve, or analytical function, is first established for the pure substance in the pure solvent for a number of known concentrations at a definite temperature. A solvent conductance correction should be used in work of precision, so that the values refer only to the conductance of the solute under consideration. The solution whose concentration is to be found is then measured conductometrically at the same temperature, and the value sought is read from the curve or computed analytically. This method for determining the concentration of pure electrolytes is capable of great accuracy and has been frequently employed.

The solubility of slightly soluble salts can be determined from conductance measurements on the saturated solutions, if the ionization is of

a simple form and complete, uncomplicated by ion complexes.²⁰ The computation is made with the equation which defines equivalent conductance:

$$\Lambda = 1000\kappa/C$$

The specific conductance, κ , is measured, and if the solubility is very low it is sufficient to use for Λ the Λ_0 value computed from the limiting ionic conductances. For precise work, a solvent conductance correction should, of course, be applied to the specific conductance determination. The solubility, C , is obtained more accurately from the equation:

$$\Lambda = \Lambda_0 - (\alpha\Lambda_0 + \beta)\sqrt{C} = 1000\kappa/C \quad (22)$$

(see Eq. 6) which can be solved by a method of approximation, or from:

$$\frac{C}{1000\kappa} = \frac{1}{\Lambda_0} + \frac{(\alpha\Lambda_0 + \beta)}{\Lambda_0^2} \sqrt{C} \quad (23)$$

which is a simple quadratic equation in \sqrt{C} .

C. PURITY OF SOLVENTS

Conductance, being a readily measurable property of solvents, can often be used to help determine the state of purity if conducting contaminants are suspected. In this connection it is useful in following the progress of removal of electrolytes. As purification proceeds, the solvent should tend to attain a constant, minimum conductance. The purity of "conductivity" water and other solvents, such as alcohol or anhydrous acetic acid, can be estimated in this way.

It is obvious, however, that the conductance method is of little use if nonconducting impurities are in question.

D. EXTENT OF HYDROLYSIS

The hydrolysis of many salts results in relatively large changes in the conductance of the solutions. For example, aniline hydrochloride hydrolyzes with the liberation of repressed, nonconducting aniline and an equivalent one of high conducting hydrogen ion. The net effect on conductance arising from this hydrolysis is due to the fact that the hydrogen ions have a greater conductance than the phenyl ammonium ions they replace in the solution.

If h is the extent of hydrolysis, the specific conductance, κ , of the hydrolyzed solution is:

$$\kappa = (1 - h)\kappa' + h\kappa_{\text{HCl}} \quad (24)$$

²⁰ F. Kohlrausch, *Z. physik. Chem.*, **44**, 197 (1903); **64**, 129 (1908). J. W. McBain and C. R. Peaker, *Proc. Roy. Soc. London*, A125, 394 (1929); *J. Phys. Chem.*, **34**, 1033 (1930).

in which κ' is the corresponding conductance of the unhydrolyzed solution and κ_{HCl} that of hydrochloric acid at the same concentration. κ' can be measured by adding enough aniline base to the salt to repress the hydrolysis. Its own ionization will be repressed by the phenyl ammonium ions present.

The extent of hydrolysis is then found from:

$$h = \frac{\kappa - \kappa'}{\kappa_{\text{HCl}} - \kappa'} \quad (25)$$

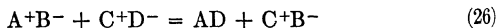
MacInnes²¹ thus computed the hydrolysis constant for aqueous solutions of aniline hydrochloride at 25° C. from Bredig's²² data:

$$K_h = \frac{h^2 C}{(1 - h)} = 2.3 \times 10^{-4}$$

IV. CONDUCTOMETRIC TITRATIONS

The addition of an electrolyte to an electrolytic solution under conditions producing no appreciable volume change will affect the conductance in a manner depending on whether ionic reactions had occurred. If no ionic interaction takes place, such as on the addition of one simple salt to another, sodium chloride to potassium nitrate, for instance, the conductance will rise. If, on the other hand, ionic interaction does occur, as on the addition of a base to an acid, the conductance will decrease, owing to the replacement of a slower metallic ion for the fast hydron. This is the principle underlying conductometric titrations.

We may write a typical ionic reaction:



in which the newly formed substance AD is relatively insoluble or only slightly ionized. As the titration proceeds, the conductivity rises or decreases depending on whether the mobility of the C^+ ion is greater or less than that of the A^+ ion. Exactly analogous considerations hold, of course, if AD remains ionized (A^+D^-), and CB is the precipitated or un-ionized substance.

During the progress of neutralizations, oxidation-reductions and precipitations, changes in conductivity may, in general, be expected, and these can, therefore, be used in determining the end points as well as the progress of the reactions. This method is particularly useful in the case of colored solutions, such as dyes, and also in cases in which potentiometric methods are not readily applicable.

²¹ D. A. MacInnes, *The Principles of Electrochemistry*. Reinhold, New York, 1939, p. 377.

²² G. Bredig, *Z. physik. Chem.*, **13**, 289 (1894).

The conductometric method of titration enjoys the advantage of being as accurate in very dilute as in more concentrated solutions. Also, hydrolysis, dissociation of the product of the reaction, and solubility do not disturb the accuracy of the method. However, large amounts of inert electrolytes have a marked effect on the accuracy attainable since the relative change in conductance is affected correspondingly.

Experimental Procedure.—The titrations are carried out in a cell whose design is not critical. A convenient form is shown in figure 14. The conductance is measured after each successive addition of reagent from a microburette has been thoroughly mixed in the cell. It is advisable to use a concentration of reagent ten or twenty times greater than that of the solution undergoing analysis in order to avoid changing the total volume appreciably.

Plots are prepared of specific conductance or reciprocal of resistance against the volume of reagent added. In general, two straight lines are observed, the intersection of which locates the end point. However, points near this intersection have no special significance. They should, in fact, often be disregarded in the construction of the lines because dissociation or solubility of the reaction product may make a contribution to the conductance which is not negligible. Such contributions should be inappreciable for the points actually used in locating the lines which determine the end points. In order to avoid errors due to temperature changes, it is best to thermostat the cell, or at least to provide for a thermometer so that rough corrections can be made, if necessary, from a knowledge of the approximate temperature coefficients of conductance for the ions present. In most work, the small volume changes during the course of titrations can be neglected, or a correction can be made by multiplying the values of reciprocal resistance, or specific conductance, by the factor $(V + v)/V$, in which V is the original volume of solution and v is the volume of reagent added.

The conductance can be measured with an alternating current Wheatstone bridge, although most of the refinements discussed in an earlier section of this chapter can usually be dispensed with. A visual form of detector is preferred by many. This can be achieved by the use of a copper-oxide or vacuum-tube rectifier, or of a thermocouple in the detector branch of the bridge, coupled to a direct current galvanometer. Another alternative is the use of ordinary 60-cycle current (of low voltage) as a source of power for the bridge, and an alternating current galvanometer as the detector.

A very simple electric circuit suitable for conductometric titrations of

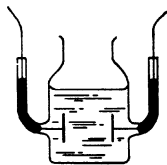


Fig. 14.—Conductance cell for titrations.

moderate accuracy is shown in figure 15a. Since we are interested merely in the change of conductance during the progress of the titration, we may follow it by observing the change in current through the cell at a constant voltage. The transformer, *T*, is of a step-down type which delivers a few

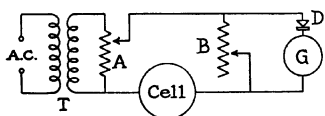


Fig. 15a.—Simple electric circuit for conductometric titrations.

vols of 60-cycle alternating current to the circuit. *D* is a rectifier and *G*, a direct current galvanometer. The variable resistances, *A* and *B*, each of a magnitude of several hundred or a thousand ohms, serve to adjust to current through the galvanometer, *G*, to a convenient initial value. With

the circuit thus adjusted, the galvanometer readings corresponding to the various additions of reagent are noted and a plot is prepared of these readings against the volume of reagent added.

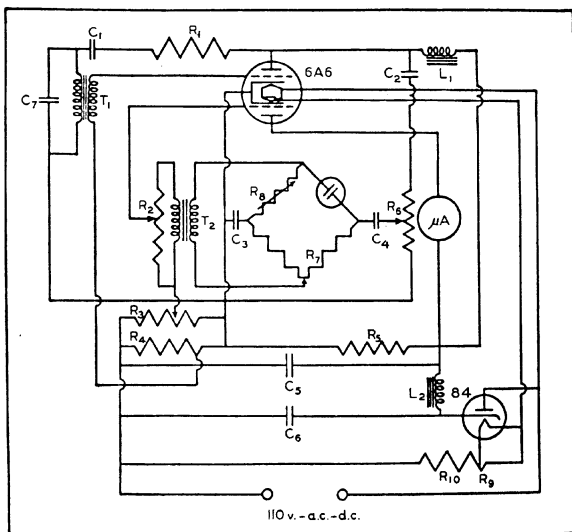


Fig. 15b.—Garman's conductivity titration circuit.²³

An excellent arrangement suitable for most conductivity titration work has been described by Garman.²³ It is a vacuum-tube circuit which contains a bridge, oscillator, and detector, and is shown diagrammatically in

²³ R. L. Garman, *Ind. Eng. Chem., Anal. Ed.*, **8**, 146 (1936).

figure 15b. The circuit can be operated from either an a. c. or d. c. 110-volt source, and is so designed that the readings on the microammeter, μA , are directly proportional to the resistance of the cell. Variable resistance R_3 serves to set the "zero point" on the meter at the beginning of titration.

Typical Conductometric Titration Applications.—Some typical conductometric curves are shown in figure 16.

When a strong acid is titrated with a strong base the conductance of the solution decreases at first, owing to the replacement of the fast hydrogen ion by a slower cation. This is seen in figure 16a,²⁴ which shows the titration of a strong acid with sodium hydroxide. The decrease in conductance ceases at the equivalence point, and further addition of base results in an increase of conductance, since the hydroxyl ions are no longer neutralized. Theoretically, the intersection point is not quite the neutral point because the mobility of the hydrogen and hydroxyl ions are not the same, but for practical purposes the difference is entirely trivial. A plot similar to that shown in figure 16a would, of course, be observed if a strong base were titrated with hydrochloric acid.

For a weak acid, such as acetic, titrated with a strong base²⁵ (or a weak base, such as ammonia, titrated with a strong acid), a plot such as is shown in figure 16b is obtained. The neutral salt, sodium acetate, which is formed during the first part of the titration tends to repress the ionization of the acetic acid still present, so that its conductance decreases. There is an increase in conductance, however, due to the rising salt concentration. These opposing effects produce curves with minima whose positions depend on the concentration, as shown in figure 16b, and on the strength of the weak acid. As the titration proceeds, a break occurs at the end point and the plot becomes linear, as was the case in figure 16a, after all the acid has been neutralized.

If the acid is very weak, for example phenol or boric acid, the initial conductance is very low and plots such as those in figure 16c are obtained if the titration is carried out with a strong base. Here again it should be pointed out that similar plots would result from the titration of a very weak base with a strong acid. The plot in figure 16c is for boric acid titrated with sodium hydroxide, taken from the work of Kolthoff.²⁵ Note that the lines are displaced slightly for different initial concentrations of the acid titrated.

A weak acid, like acetic acid, can also be titrated satisfactorily with a weak base, like ammonia. The initial portion of the curve is similar to the one obtained with sodium hydroxide. After the end point, however, the further addition of ammonia produces no appreciable change in con-

²⁴ W. Poethke, *Z. anal. Chem.*, **86**, 45 (1931).

²⁵ I. M. Kolthoff, *Ind. Eng. Chem., Anal. Ed.*, **2**, 225 (1930).

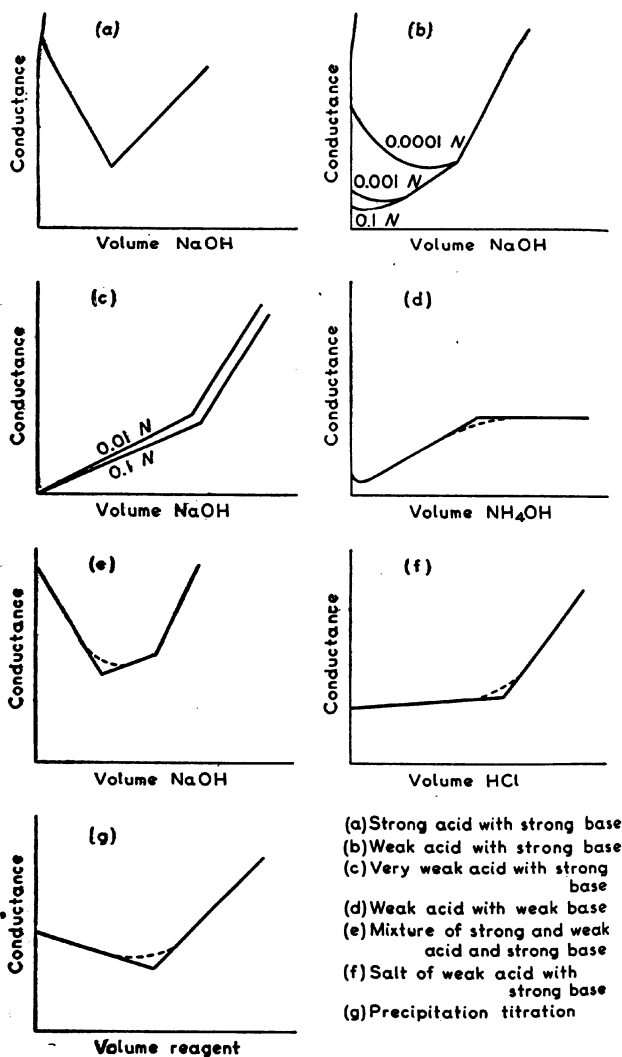


Fig. 16.—Conductometric titration curves.

ductance since its ionization is depressed by the ammonium salt which had been formed. A plot for 0.01 *N* acetic acid titrated with 0.1 *N* ammonium hydroxide is shown in figure 16d, in which the dotted portion, near the end point, is neglected in the construction of the intersecting lines for reasons which had been discussed previously. That is, the points near the end point are affected by hydrolysis which, in this case, result in lower conductance values. Acids much weaker than acetic, such as boric acid or phenol, cannot be satisfactorily titrated with ammonia because the hydrolysis error extends through most of the region of the plot.

Conductometric titration is particularly useful in the titration of mixtures of acids or bases, solutions which often cannot be satisfactorily analyzed by the usual potentiometric methods. Figure 16e shows the conductivity titration plot of a mixture of hydrochloric and acetic acids, each at about 0.01 *N*, with sodium hydroxide as the reagent (Kolthoff). The first break in the plot corresponds to the end point for the strong acid and the second to the one for the weak acid. If hydrolysis effects, shown by the dotted line, extend too far to establish the middle linear branch without ambiguity, a better result can be obtained by adding ethyl alcohol to the solution at the start of the titration.

The plot shown in figure 16f is typical of titrations in which a salt of a weak acid is titrated with a strong acid, or a salt of a weak base is titrated with a strong base. Here again it is frequently advantageous to add alcohol to the solution in order to decrease the ionization of the weak liberated acid (or base), thus minimizing the errors near the end point indicated by the dotted lines. In the case shown, sodium acetate is titrated with hydrochloric acid. The first portion of the plot shows but little rise in conductance due to the liberated weak acid whose ionization tends to be repressed by its salt. After the end point, the conductance rises more sharply due to the increasing concentration of free strong acid.

Conductometric analysis can also be used in many precipitation and complex formation reactions. Plots such as that in figure 16g are obtained in favorable cases. However, the results tend to be less accurate than in the other instances discussed because of complications arising from slow rate of precipitation, adsorption, instability of complexes, and other factors characteristic of special cases.

For the organic chemist, conductivity titrations will be found useful in the analysis of hydroxybenzenes, phenols, particularly some of its colored derivatives, amino acids, polypeptides, proteins, vanillin, aniline, pyridine, sulfophthaleins, alkaloids, etc.

The literature should be consulted for specific applications which are too numerous to cite in this review. A number of references are given in the

appendix. Kolthoff²⁶ and Kolthoff and Laitinen²⁷ include many more references; and their books will be helpful in supplying the reader with details which could not be given in this chapter on conductometry without placing undue emphasis on conductivity titrations.

V. APPENDIX*

LIMITING ION CONDUCTANCES IN WATER AT 25° C. AND APPROXIMATE
TEMPERATURE COEFFICIENTS

$$\lambda_i^\circ = \lambda_{25}^\circ [1 + a(t - 25)]$$

Cation	λ_{25}°	a	Anion	λ_{25}°	a
K ⁺	73.52	0.0189	Br ⁻	78.4	0.0187
Na ⁺	50.11	0.0209	I ⁻	76.8 ₆	0.0186
H ⁺	349.82	0.0142	NO ₃ ⁻	71.44	0.0180
Ag ⁺	61.92	0.0197	HCO ₃ ⁻	44.48
Li ⁺	38.69	0.0226	OH ⁻	198	0.0160
NH ₄ ⁺	73.4	0.0192	CH ₃ CO ₂ ⁻	40.9
Tl ⁺	74.7	0.0187	CH ₂ ClCO ₂ ⁻	39.7
1/2 Ca ⁺⁺	59.50	0.0211	CH ₃ CH ₂ CO ₂ ⁻	35.81
1/2 Ba ⁺⁺	63.64	0.0206	CH ₃ (CH ₂) ₂ CO ₂ ⁻	32.59
1/2 Sr ⁺⁺	59.46	0.0211	ClO ₄ ⁻	68.0
1/2 Mg ⁺⁺	53.06	0.0218	C ₆ H ₅ CO ₂ ⁻	32.3
1/2 La ⁺⁺⁺	69.6	1/2 SO ₄ ⁻	79.8	0.0196
1/2 Co(NH ₃) ₆ ⁺⁺⁺	102.3	1/2 Fe(CN) ₆ ⁻⁻⁻	101.0
Cl ⁻	76.34	0.0188	1/4 Fe(CN) ₆ ⁻⁻⁻	110.5

LIMITING EQUIVALENT CONDUCTANCES FOR SOME ELECTROLYTES
IN METHYL ALCOHOL AT 25° C.

Salt	Λ_0	Salt	Λ_0
LiCNS	101.8	CaCl	113.6
NaCNS	107.0	LiNO ₂	100.2
KCNS	114.5	NaNO ₂	106.4
RbCNS	118.2	KNO ₂	114.5
CsCNS	123.2	RbNO ₂	118.1
NH ₄ CNS	118.7	CsNO ₂	122.9
LiCl	90.9	Mg(CNS) ₂	120
NaCl	96.9	Ca(CNS) ₂	122
KCl	105.0	Sr(CNS) ₂	122
RbCl	108.6	Ba(CNS) ₂	125

* Data for the four tables in the Appendix are taken in part from D. A. MacInnes, *Principles of Electrochemistry*, Reinhold, 1939, pages 342, 357-358, 339, and 349, respectively.

²⁶ I. M. Kolthoff, *Konduktometrische Titrationen*. Steinkopff, Dresden, 1923.

²⁷ I. M. Kolthoff and H. A. Laitinen, *pH and Electro Titrations*. Wiley, New York, 1941.

EQUIVALENT CONDUCTANCES OF SOME ELECTROLYTES IN WATER AT 25° C.

Electrolyte	Concentration, equivalents per liter								Reference
	0.0000	0.0005	0.001	0.005	0.01	0.02	0.05	0.10	
NaCl	126.45	124.50	123.74	120.65	118.51	115.76	111.06	106.74	a
KCl	149.86	147.81	146.95	143.55	141.27	138.34	133.37	128.96	a
LiCl	115.03	113.15	112.40	109.40	107.32	104.65	100.11	95.86	b, c
HCl	426.16	422.74	421.36	415.80	412.00	407.24	399.09	391.32	b
NH ₄ Cl	149.7	141.28	138.33	133.29	128.75	d, e
KBr	151.9	146.09	143.43	140.48	135.68	131.39	f
KI	150.3 _a	144.37	142.18	139.45	134.97	131.11	d
NaI	126.94	125.36	124.25	121.25	119.24	116.70	112.79	108.78	g
NaO ₂ CCH ₃	91.0	89.2	88.5	85.72	83.76	81.24	76.92	72.80	h, d
NaO ₂ CCH ₂ CH ₃	85.92	84.24	83.54	80.90	79.05	76.63	i
NaO ₂ C(CH ₂) ₂ CH ₃	82.70	81.04	80.31	77.58	75.76	73.39	69.32	65.27	i
KNO ₃	144.96	142.77	141.84	138.48	132.82	132.41	126.31	120.40	b
KHCO ₃	118.00	116.10	115.34	112.24	110.08	107.22	f
AgNO ₃	133.36	131.36	130.51	127.20	124.76	121.41	115.24	109.14	b
NaOH	248	246	245	240	237	233	227	221	k
1/2 CaCl ₂	135.84	131.93	130.36	124.25	120.36	115.65	108.47	102.46	i
1/2 BaCl ₂	139.98	135.96	134.34	128.02	123.94	119.09	111.48	105.19	i
1/2 SrCl ₂	135.80	131.90	130.33	124.24	120.29	115.54	108.25	102.19	i
1/2 MgCl ₂	129.40	125.61	124.11	118.31	114.55	110.04	103.08	97.10	i
1/2 Ca(OH) ₂	258 ¹⁷	233	226	214	m
1/2 Na ₂ SO ₄	129.9	125.74	124.15	117.15	112.44	106.78	97.75	89.98	n, o
1/2 NiSO ₄	118.7	113.1	93.2	82.7	72.3	59.2	50.8	p
1/4 LaCl ₃	145.9	139.6	137.0	127.5	121.8	115.3	106.2	99.1	q
1/4 K ₄ Fe(CN) ₆	184	167.24	146.09	134.83	122.82	107.70	97.87	r

^a T. Shedlovsky, A. S. Brown, and D. A. MacInnes, *Trans. Electrochem. Soc.*, **66**, 165 (1934).

^b T. Shedlovsky, *J. Am. Chem. Soc.*, **54**, 1411 (1932).

^c D. A. MacInnes, T. Shedlovsky, and L. G. Longworth, *ibid.*, **54**, 2758 (1932).

^d L. G. Longworth, *private communication*.

^e L. G. Longworth, *J. Am. Chem. Soc.*, **57**, 1185 (1935).

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^g R. A. Lasselle and J. G. Aston, *J. Am. Chem. Soc.*, **55**, 3067 (1933).

^h D. A. MacInnes and T. Shedlovsky, *ibid.*, **54**, 1429 (1932).

ⁱ D. A. Belcher and T. Shedlovsky, *private communication*.

^j T. Shedlovsky and D. A. MacInnes, *J. Am. Chem. Soc.*, **57**, 1705 (1935).

^k G. H. Jeffery and A. I. Vogel, *Phil. Mag.*, **17**, 582 (1934); M. Randall and C. C. Scallione, *J. Am. Chem. Soc.*, **49**, 1486 (1927); J. Goworecka and M. Hlasko, *Roczniki Chem.*, **12**, 403 (1932).

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^m T. Noda and A. Miyoshi, *J. Soc. Chem. Ind. Japan*, **35**, Suppl. Bndg., 317 (1932);

F. M. Lea and G. E. Bessey, *J. Chem. Soc.*, 1937, 1612.

ⁿ From sum of ion mobilities.

^o T. Shedlovsky, *private communication*.

^p K. Murata, *Bull. Chem. Soc. Japan*, **3**, 47 (1928).

^q G. Jones and C. E. Bickford, *J. Am. Chem. Soc.*, **56**, 602 (1934).

^r G. Jones and F. C. Jelen, *ibid.*, **58**, 2561 (1936).

IONIZATION CONSTANTS OF ACIDS AT 25° C. FROM CONDUCTANCE MEASUREMENTS

Acid	$K \times 10^4$	Ref.	Acid	$K_1 \times 10^4$	Ref.
Acetic	1.753	^a	Carbonic	0.0431	^k
Monochloroacetic	139.6	^b	Malonic	139.7	^l
Propionic	1.343	^c	Succinic	6.63	^l
<i>n</i> -Butyric	1.506	^c	Glutaric	4.54	^l
Benzoic	6.30	^{d-f}	Adipic	3.72	^l
<i>o</i> -Chlorobenzoic	119.7	^e	Pimelic	3.10	^l
<i>m</i> -Chlorobenzoic	15.06	^e	Suberic	2.99	^l
<i>p</i> -Chlorobenzoic	10.4	^e	Methylmalonic	8.47	^m
<i>o</i> -Bromobenzoic	140	^e	Ethylmalonic	10.9	^m
<i>p</i> -Bromobenzoic	10.7	^e	<i>n</i> -Propylmalonic	10.3	^m
<i>p</i> -Fluorobenzoic	7.22	^e	Dimethylmalonic	7.06	^m
Phenylacetic	4.88	^g	Methylethylmalonic	15.4	^m
<i>o</i> -Chlorophenylacetic	8.60	^f	Diethylmalonic	70.8	^m
<i>m</i> -Chlorophenylacetic	7.24	^f	Ethyl- <i>n</i> -propylmalonic	78.4	^m
<i>p</i> -Chlorophenylacetic	6.45	^h	Di- <i>n</i> -propylmalonic	92.0	^m
<i>o</i> -Bromophenylacetic	8.84	^f	Phenylmalonic	277	ⁿ
<i>p</i> -Bromophenylacetic	6.49	^h	Cyclopropane-1,1-di-	150	^o
<i>p</i> -Iodophenylacetic	6.64	^h	carboxylic		
<i>p</i> -Methoxyphenylacetic	4.36	^f	Cyclobutane-1,1-di-	7.55	^o
Acrylic	5.50	ⁱ	carboxylic		
Lactic	13.87	^j	Cyclopentane-1,1-di-	5.96	^o
			carboxylic		
			Cyclohexane-1,1-di-	3.54	^o
			carboxylic		

^a D. A. MacInnes and T. Shedlovsky, *J. Am. Chem. Soc.*, **54**, 1429 (1932).

^b B. Saxton and T. W. Langer, *ibid.*, **55**, 3638 (1933); T. Shedlovsky, A. S. Brown, and D. A. MacInnes, *Trans. Electrochem. Soc.*, **66**, 165 (1934).

^c D. Belcher, *J. Am. Chem. Soc.*, **60**, 2744 (1938).

^d F. G. Brockman and M. Kilpatrick, *ibid.*, **56**, 1483 (1934).

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^f J. F. C. Dippy and F. R. Williams, *ibid.*, **1934**, 1888.

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^h J. F. C. Dippy and F. R. Williams, *ibid.*, **1934**, 161.

ⁱ W. I. German, G. H. Jeffery, and A. I. Vogel, *ibid.*, **1937**, 1604.

^j A. W. Martin and H. V. Tartar, *J. Am. Chem. Soc.*, **59**, 2672 (1937).

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ⁿ S. Basterfield and J. W. Tomecko, *Can. J. Research*, **8**, 447 (1933).

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ELECTROPHORESIS

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I. INTRODUCTION

The utility of electrophoresis to the organic chemist has developed largely since the advent of the *Tiselius apparatus* in 1937. Although the *microscopic method* and earlier *moving-boundary techniques* have served and still are serving in the field of organic chemistry, this chapter will therefore concern itself principally with a description of the Tiselius apparatus with its various modifications and a critical outline for its judicious use. It is intended to acquaint the reader with the entire process of using a Tiselius electrophoresis apparatus, pointing out the many pitfalls and erroneous conclusions which may be drawn from results.

II. GENERAL

Electrophoresis or cataphoresis may be defined as the migration of dissolved or suspended particles under the influence of an electric field. The

first recorded measurements of this phenomenon were made by Quincke in 1861, although years before (1808) Reuss had observed the flow of water through clay (electro-osmosis) due to an electric current and also the migration of clay in the opposite direction. The most important early theoretical studies in electrokinetics were made by Helmholtz whose papers published in 1879–1888 furnished the groundwork for present concepts. Other theoretical workers were Smoluchowski, Gouy, Debye, Hückel, Abramson, and MacInnes.

When suspended in an aqueous solvent, practically all particles (*e. g.*, bacteria, red blood cells, protein molecules; etc.) acquire either a positive or negative charge depending on the nature of the particle and the solvent. Protein molecules, for example, have large numbers of free amino and carboxyl groups on their surfaces. These groups determine the net charge density of the protein molecule which causes it to migrate in an electric field in a direction and at a velocity dependent on the sign and quantity of this net charge density. The net charge, however, is not fixed but may be varied by changing the *pH* or ionic strength of the solvent. The greater the concentration of H ions in the solvent the more positively charged the molecule will tend to be. Thus, to measure the mobility of a particle, it is necessary to maintain constant ionic conditions. Moreover, the particle is constantly taking on and giving up charges so that, while the instantaneous charge is an integral multiple of an electronic charge, the time-average charge may be fractional. The fractional nature of the net charge makes possible a larger number of electrophoretic identifications at a given *pH* than would be possible if the net charge were a multiple of only whole unit charges.

Introduction of a charged colloidal particle into a homogeneous electrolyte causes a redistribution of the ions. Suppose that the particle is spherical and carries a negative charge; there will then be a concentration of positive ions in the region surrounding the particle in order to make the total charge zero. Helmholtz assumed that these outer positive ions were concentrated in a single layer at a definite distance from the surface, thus creating a potential difference between the particle surface and the outer layer. The Gouy-Debye theory shows that the outer layer consists of a diffuse ionic atmosphere in which the charge density increases rapidly near the surface and approaches zero asymptotically at an infinite distance from the surface, as illustrated in figure 1. In this diagram the stippled area represents a region where solvent ions are bound so firmly to the surface that they move with it to form the shear boundary. The potential, ψ , at any point outside the shear boundary is indicated by the curve. The thickness of the diffuse double layer is defined as that distance of separation $1/\kappa$ of

an equivalent Helmholtz layer of charge Q which would produce the same potential drop as exists in the diffuse layer.

The potential difference between the particle of radius r_1 and the assumed Helmholtz layer of radius r_2 is:

$$V_1 - V_2 = \int_{r_1}^{r_2} \frac{Q}{Dr^2} dr = \frac{Q(r_2 - r_1)}{Dr_1r_2}$$

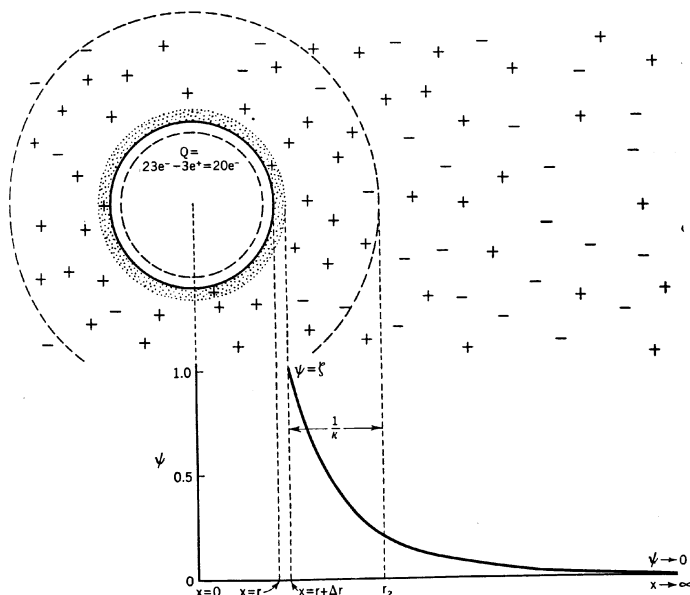


Fig. 1.—Diagram showing spherical charged particle surrounded by ionic double layer. Values of ψ are in relative units and are negative.¹

where Q is the charge on the particle and D is the dielectric constant. This difference in potential is known as the ζ potential. It is a function of the thickness of the double layer and is inversely proportional to the dielectric constant. If electrodes are placed in the electrolyte on each side of the particle under consideration, one electrode being made positive and the

¹ H. A. Abramson, L. S. Moyer, and M. H. Gorin, *Electrophoresis of Proteins and the Chemistry of Cell Surfaces*. Reinhold, New York, 1942.

other negative, the particle will tend to migrate with a velocity given by Stokes' law:

$$V = EQ/6\pi\eta$$

where E is the impressed field in volts per centimeter and η is the viscosity. Due to backward drag of the ionic double layer, which tends to migrate in the direction opposite to the particle, the latter will not migrate with the Stokes' velocity but with some smaller velocity given by $V = DE\xi/6\pi\eta$ for a particle of such size and shape that the distortion of the electric field due to the particle is negligible. For larger particles the maximum velocity is given by:

$$V = DE\xi/4\pi\eta$$

Although distortion of the field would seem to depend on the shape of the particle, Abramson^{1a} has shown that the velocity of migration of protein-covered particles and of particles having the same density of net charge is independent of shape. The work of Mooney² on oil droplets has shown that over a wide variety of sizes the velocity in the systems he studied may depend on size. At present the theory is incomplete due to lack of knowledge of the physical conditions prevalent in the immediate neighborhood of the surface of the particle and therefore the equations remain only of theoretical interest.

Electrophoresis may be used: (a) analytically to determine the relative concentration of colloidal components in mixtures as well as to determine certain physical properties which influence the rate of migration in an electric field; or (b) it may serve as a means for the *separation* of particles which are differentiated in their electrophoretic behavior. Both aspects are treated in the present chapter.

III. MICROSCOPIC METHOD

1. Theory

Movement of particles under the influence of an electric field may be observed and measured with ordinary microscopic equipment, provided the particles are large enough to be visible by this means. Invisible substances, such as proteins in solution, will adhere to and coat visible particles of quartz or collodion and impart to them the mobility of the protein. If a mixture of proteins is used, however, an average mobility will be obtained and this average value may be dependent on the affinity between each species and the quartz. This is of practical importance because of

^{1a} H. A. Abramson, *J. Phys. Chem.*, **35**, 289 (1931).

² A. Mooney, *J. Phys. Chem.*, **35**, 331 (1931).

the difficulty of obtaining pure proteins. Nevertheless, many valuable studies have been made by the method.

A special cell is placed on the stage of a microscope and the velocity and orientation of the particle in a given electric field strength are observed. Reuss and Quincke showed that in narrow cells there is a flow of the solvent liquid known as electro-osmotic flow. This is in one direction near the wall of the tube and in the opposite direction along the axis of the closed tube, thereby obviously interfering with the independent migration of the particle. Therefore, in order to get the true migration of a particle, a correction dependent on the position of the particle in the micro electrophoresis cell must be applied. The theory for the streaming of liquid in cells has been worked out by Smoluchowski.

2. Apparatus

One of the most satisfactory electrophoresis cells was designed by Northrop and Kunitz.³ This cell,⁴ as modified by Abramson, is shown in figure

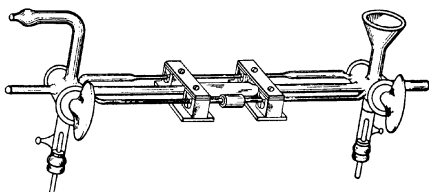


Fig. 2.—Microscopic electrophoresis cell.

2. It is constructed of one piece of glass and can readily be cleaned with alkali, acid, or cleaning fluid. The instrument rests directly on the stage of the microscope and can be inverted to resuspend settled particles. Construction of the side arms permits introduction of plaster-of-paris plugs into the electrode vessels. These plugs prevent precipitation of protein by diffusion of the copper sulfate from the electrode compartment into the side arm. Because of nonpolarizability of the electrodes the technique is well adapted for use in biologic systems.

A vertical model of this cell is also available. In both types of cell the field strength can readily be computed by applying Ohm's law. Another method suitable for quantitative development has been described by Smith and Lisse.⁵ Their cell has a side tube of smaller diameter in parallel with

³ J. H. Northrop and M. Kunitz, *J. Gen. Physiol.*, **7**, 729 (1925).

⁴ Obtainable from Kopp and Staub Glass Instrument Co., New York, N. Y.

⁵ M. E. Smith and M. W. Lisse, *J. Phys. Chem.*, **40**, 399 (1936).

the observation cell which greatly reduces and probably eliminates correction for electro-osmotic flow.

IV. EARLY MOVING-BOUNDARY APPARATUS

The development of the moving-boundary method of electrophoresis came about by a long series of improvements made by numerous investigators. A technique for removing different constituents separated by an electric field was devised by Ottenberg and Stenbuck.⁶ This apparatus, however, did not form suitable boundaries, a difficulty partly overcome by Theorell,⁷ who separated a U tube into various compartments by means of disks, so that analysis could be made of the constituents of each compartment. Until Tiselius⁸ described his new electrophoresis cell (1937), many of the early difficulties of the moving boundary method were still present. These difficulties were: (1) convection resulting from heat generated by the electric current flowing through the cell; (2) small and too similar electrical mobilities of most colloids, thus necessitating long tubes for separating them; and (3) observation of the various components after separation. The apparatus of Tiselius has largely overcome these difficulties.

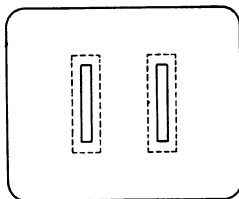


Fig. 3.—Flange plate of Tiselius cell.

V. TISELIUS MOVING-BOUNDARY METHOD

1. Cells

A. ANALYTICAL CELLS

Probably the most important element of the Tiselius apparatus is the unique type of cell used.* Tiselius designed a U tube of rectangular rather than circular cross section, composed of several segments having flanged plates at each end (see Fig. 3) which can be greased and butted against each other to form leakproof seals. Segregated compartments can then be formed simply by displacing sections horizontally. This permits the formation of sharp boundaries and also the segregation of components after migration. The rectangular tube with a large surface area increases dissipation of heat, and if used in a thermostatic bath a little under 4° C., where change of density with temperature is minimal, comparatively large currents may be used without convection. The cells must not be made too

* Obtainable from *Pyrocell Manufacturing Co.*, New York.

⁶ R. Ottenberg and F. A. Stenbuck, *J. Gen. Physiol.*, **9**, 345 (1925).

⁷ H. Theorell, *Biochem. Z.*, **278**, 291 (1935).

⁸ A. Tiselius, *Trans. Faraday Soc.*, **33**, 524 (1937).

narrow, however, lest fluid flow resulting from electro-osmosis and the viscous drag along the cell wall of migrating components become appreciable. It has been found that these effects do not become apparent with cell widths down to 1.5 mm., but smaller widths are not recommended. Cells of 2 to 3 mm. have been used most widely for analytical work. Separatory cells may be much wider but little is gained by making them wider than 7 to 10 mm., because of inadequate heat dissipation, thus requiring smaller current densities and slower separation. The cell dimension along the optic path also has limitations for best analytical results. Since detection and measurements of boundary gradients depend on the bending of the

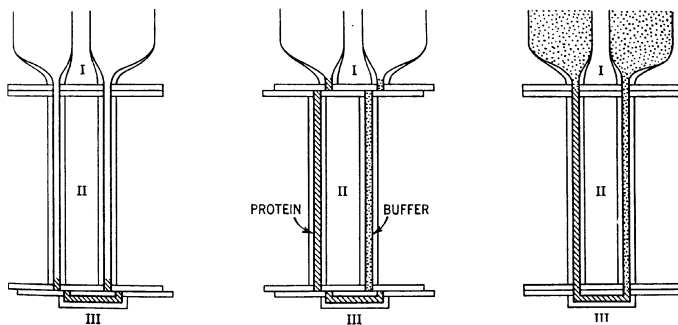


Fig. 4.—A Tiselius cell; the three steps in the filling process are indicated.

light rays which traverse the cell (see page 1697) and the bending is proportional to the distance through the cell, it is obvious that the sensitivity of an apparatus is a direct function of this cell dimension. The fidelity with which the true refractive-index gradients are recorded, decrease, however, with increasing cell thickness. This infidelity results from the fact that rays start being deflected by a refractive-index gradient as soon as they enter the cell. Rays which traverse the cell above the position of maximum gradient (usually the center of the boundary) are deflected into regions of greater gradient which deflect them still more, whereas rays which enter the cell below the center of a boundary are deflected into a region of less gradient. This tends to make the pattern of the boundary skewed by an amount which is a function of the sharpness of the boundary. The effect is negligible, however, with the cells and boundaries dealt with in general practice. Svensson⁹ reports that when light was made to traverse a cell (25 mm.) twice by placing a mirror 3.3 cm. behind it, a sharp boundary

⁹ H. Svensson, *Arkiv Kemi Mineral. Geol.*, A22, No. 10, 102 (1946).

appeared double in the pattern. Light should not enter the cell horizontally but should be inclined upward slightly, so that the undeviated rays fall a little above the optic axis and the deviated rays below it.

The process of filling a cell may be understood by the aid of figure 4. The filling is accomplished with a syringe and needle long enough to reach the bottom of the cell. The bottom section III is first filled with the colloidal solution with a slight excess extending up into the channels of section II (to assure complete filling of the bottom section). Section III is then segregated by shifting section II to the right. The left channel of section II is filled with the colloidal solution again with a slight excess extending up into section I. After the small amount of excess colloidal solution is removed from the right-hand limb of section II, it is then thoroughly rinsed

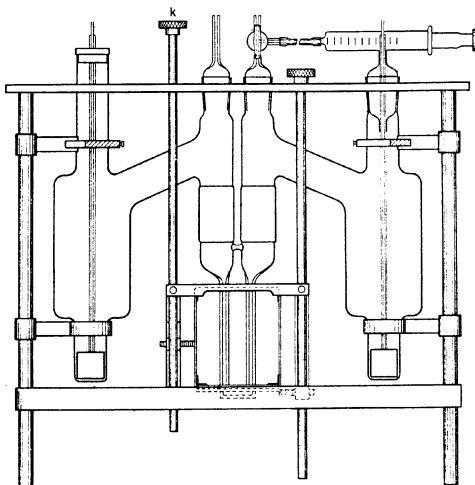


Fig. 5.—Cell assembly with one side closed.¹⁰

and overfilled with buffer. Section II is then segregated from section I by shifting section I to the left. The left-hand limb of the top section is then rinsed free of colloid. It has been found advantageous to have the cell precooled in the icebox before this process is begun, although it is not essential. After the cell is filled the buffer tubes are connected and filled with buffer solution.

The entire assembly is then ready to be placed in the thermostat. The filling process may be carried out so quickly that the cell and its contents do not have a chance to become warm. The silver-silver chloride electrodes are now placed in the electrode vessels and chilled one-third saturated potassium or sodium chloride solution sufficient to cover the electrodes is slowly passed through the lead tubes.

The boundaries are formed by shifting the center section to alignment by turning the knob *k*. Before the current is switched on, the boundaries are slowly shifted out into view and photographed, thus recording the starting position for mobility measurements.

In order to obtain more complete separation before any component migrated out of the cell, Tiselius devised a method for causing the entire contents to flow in the opposite direction to that of the electrophoretic migration. This may be done by slowly lowering a plunger by means of a clock-work mechanism into the buffer tube toward which the migration is progressing, or by slowly flowing fresh buffer into the same buffer tube with a mechanically controlled syringe. A modification (Fig. 5) of the original

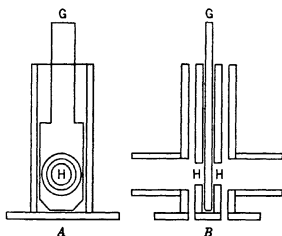


Fig. 6.—Top section for 2-cc. Tiselius cell with ground-glass gate. G: (A) lateral view; (B) front view.

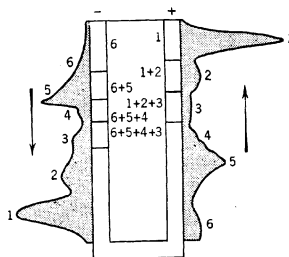


Fig. 7.—Schematic electrophoresis cell indicating the way in which protein components may be separated.¹²

Tiselius cell assembly has been described by Longworth and MacInnes.¹⁰ In their apparatus one of the buffer tubes is closed; closing one side adds stability to the system. Compensation is achieved by forcing buffer either into or out of the closed side. A gated top section (illustrated in Fig. 6) for obtaining instantaneous hydrostatic equilibrium with the open electrode system has been used by the author.¹¹ When the gate, *G*, is raised, buffer may flow freely from one side to the other through the hole, *H*.

Since electrophoretic migration of an amphoteric ion depends on the *pH* of the solvent, it is important to maintain a constant *pH* on each side of the boundary throughout the entire experiment; also, the conductivity should be held constant and no ions from the electrodes should ever reach the colloid solution. Consequently, it is necessary to have a large volume of suitable buffer between boundaries and electrodes. To prevent formation of gas at the electrode and to minimize volume changes by electrolysis.

¹⁰ L. G. Longworth and D. A. MacInnes, *Chem. Revs.*, **24**, 271 (1939).

¹¹ D. H. Moore and J. U. White, *Rev. Sci. Instruments*, **19**, 700 (1948).

silver-silver chloride electrodes are used. These electrodes are placed at the bottom of the buffer tubes as far away as possible from the solution under investigation. Leads to the electrodes pass down through the buffer by way of insulated tubes and a saturated solution of potassium chloride or sodium chloride is layered around the electrodes underneath the buffer.

B. SEPARATION CELLS

Recovery of separated electrophoretic fractions may be made in any Tiselius cell. Only two components, the one with highest mobility and the one with lowest mobility, can be recovered in pure form. The way in which samples may be recovered is illustrated¹² in figure 7.

Here samples of components 1, 1 + 2, and 1 + 2 + 3 were removed from the ascending limb of the cell and samples of components 6, 6 + 5, 6 + 5 + 4, and 6 + 5 + 4 + 3 from the descending limb. This procedure involves several steps. After the components had migrated within the limits of the cell their positions were recorded by photographing the boundaries; the compartments of the cell were segregated by displacing one of the sections. The assembly was removed from the bath and the buffer was aspirated from the top section (section I of Fig. 4). The samples were recovered with a blunt needle and syringe which was introduced barely below the meniscus following it down with a slow to and fro movement from one end of the cell to the other. The pattern served as a guide, of course, in determining the position of each boundary. Great care must be taken not to stir or cause any mixing below the meniscus. Density gradients existing at each boundary help to stabilize the system, but if the concentration change at the boundary is small, their stabilizing effect is proportionately less.

Usually the active component can be located quite precisely in the cell if great care is taken. For example, in the sample of fowl serum illustrated in figure 7 the specific antibody sought was found to be associated with component 4. In the process of purifying highly active substances the activity may sometimes be found to be associated with a component having a concentration lower than the optical sensitivity of the apparatus. By removing sufficiently small samples, however, the approximate mobility of the active component may be established. This sometimes serves as a considerable aid in the process of chemical purification. Furthermore, the relative amounts of active and inert fractions may be evaluated, thereby indirectly permitting an evaluation of the procedure used to isolate the desired material. For most precise determinations activity should be correlated with nitrogen content, dry weight, spectroscopic absorption, refractivity, or other such procedures. A more precise determination of the

¹² H. Koprowski, G. Richmond, and D. H. Moore, *J. Exptl. Med.*, **85**, 515 (1947).

boundary position may be made in the ascending limb because there may be some adherence to the cell wall in the descending limb.

If it is desired to recover components other than those of highest and lowest mobilities in pure form multiple separations are required. For instance, if a sample of component 4 (Fig. 7) free of all other components were desired, the sample containing components 6 + 5 + 4 recovered from a first electrophoretic separation could be re-electrolyzed in a smaller cell, whereupon component 4 would have the highest mobility, thus permitting recovery of a pure though small sample.

2. Use of Thermostat

The passage of current through an electrophoresis cell generates heat which will result in convection and destruction of the boundaries if it is permitted to produce an appreciable change in the density of the contents

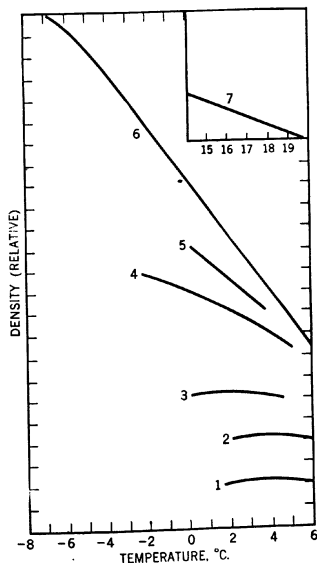


Fig. 8.—Density-temperature curves for some common aqueous solutions: (1) water; (2) 0.05 *M* lithium barbiturate + 0.025 *M* LiCl; (3) 0.15 *M* NaCl + 0.02 *M* sodium phosphate; (4) 1.0 *M* urea; (5) 1.8 *M* sucrose; (6) 2.8 *M* urea + 0.15 *M* NaCl + 0.02 *M* sodium phosphate; (7) water (slopes and not positions of curves are significant).¹³

¹³ D. H. Moore, *J. Am. Chem. Soc.*, **64**, 1090 (1942).

of the cell. Tiselius minimized this effect by operating the cell in a thermostat set at a temperature slightly below the temperature of maximum density (t.m.d.) of the solution. The addition of solute depresses the t.m.d. If, therefore, concentrated solutions are employed, the thermostat temperature must be lowered to avoid convection. Variation of density with temperature for some common solutions is illustrated¹³ by the curves of figure 8.

The selection of thermostat temperature depends also on the thermal conductivity of the solution and of the glass cell wall. Since heat is dissipated by the cell wall the warmest place in the system is along the central

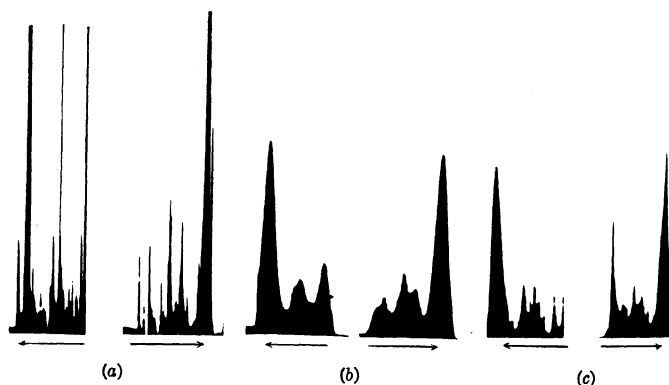


Fig. 9.—Electrophoresis patterns of human serum in the presence of 2.8 *M* urea in a temperature bath at 1.5°; left ascending pattern, right descending pattern: (a) after 4.8 volts per cm. had been applied for four hours; (b) after standing for twelve additional hours without voltage; (c) after voltage was applied for an additional thirty minutes, then switched off for one hour.¹³

axis of the cell. The temperature of the thermostat should be such that the temperature of the inside cell wall is as far below the t.m.d. of the solution as the solution in the center of the cell is above it. This ideal thermostat temperature is, of course, not only a function of the solution and cell used but also of the current or rate of heat generation in the cell. Fortunately, however, the density-temperature curves are rather flat in the region of t.m.d. so that the setting is not too critical. A thermostat temperature of from 0° to 1° C. is satisfactory for the usual conditions of an experiment. The regulation should be much better than 1° C., however, for precise mobility determinations, since the electrical conductivity of solutions is a sensitive function of temperature (see Chapter XXV).

The maximum current which may be employed without convection has been investigated by Alvarez-Tostado.¹⁴ Boundaries having large density gradients reduce the disturbing effects of convection, whereas small boundaries are easily disturbed and require lower maximum currents.

Thermal convection usually manifests itself by producing spiked patterns such as those illustrated in figure 9. The sharp gradients disappear upon standing but the true shape of the boundary may have been irreparably marred.

3. Optics

The optical methods in general use in analytical electrophoresis depend upon the refractive-index gradients formed at boundaries. These refractive index methods are of two types: (1) the Lamm scale method, and (2) the Toepler Schlieren method. A variety of modifications of these methods have been discussed in Chapter XII. Only some improvements and comparative data on the Schlieren scanning method of Longworth,¹⁵ which is believed to hold the greatest possibilities for accuracy and convenience,

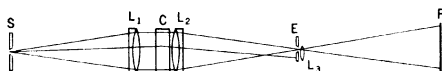


Fig. 10—Optical arrangement using two Schlieren lenses and two scanning edges.

will be presented here. Methods using cylindrical lenses and diagonal edges have the advantage of convenience in making refractive-index gradient curves directly visible on the ground-glass screen. They have the disadvantage, however, in the uncertainty in the position of the tracing line because the diffraction pattern of the inclined edge of slit is not parallel to the cell. Moreover, properly corrected cylindrical lenses are difficult to obtain.

In the Schlieren scanning method of Longworth the light passing through the electrophoresis cell is slightly converging. Parallel light through the cell may be obtained by moving the source from twice the focal distance of the Schlieren lens (a Schlieren lens is any lens used in such a manner to detect refractive-index gradients) up to its focal plane and placing another Schlieren lens, L_2 , of the same focal length on the other side of the cell C , as is illustrated in figure 10. The distance to the focal plane at E is now reduced to one half its original value. This permits a reduction

¹⁴ C. Alvarez-Tostado, *J. Biol. Chem.*, **135**, 799 (1940).

¹⁵ L. G. Longworth, *J. Am. Chem. Soc.*, **61**, 529 (1939).

also in the focal length of the camera lens, L_3 . If, however, the Schlieren lenses have too large an aperture and are separated by too great a distance, coma may result from lateral spherical aberration.

Due to diffraction effects at the cell and at the scanning diaphragm, the exact pattern edge is rendered indeterminate. Consequently, the apparent pattern edge becomes a function of light exposure. Since most biological fluids contain light-absorbing components which make it impossible to maintain constant uniform exposure, this becomes an important consideration which will become evident from figure 11. This pattern was taken

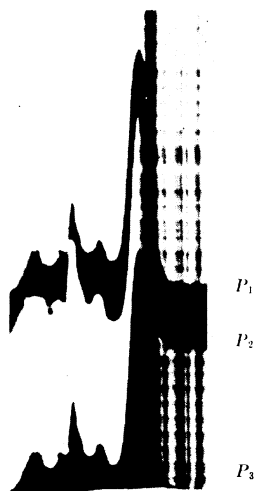


Fig. 11.—Scanning patterns (descending) made by a scanning rod or bar (P_1 and P_2) and by an edge (P_3), the latter being a standard Longworth pattern.

after fastening a 2-mm. rod to the scanning knife edge (see Fig. 22, Chapter XII) at a position about 7 mm. above it. Upon scanning, three patterns appeared; the one at the bottom, P_3 , was produced by the usual edge and the other two, P_1 and P_2 , by the upper and lower edges of the scanning rod. The pattern shown in figure 11 (a descending pattern) was of human plasma in which the albumin as well as the globulins absorbed light. The absorption caused the shadow cast by the rod to be nonuniform in width. In the original pattern the vertical width (distance between P_1 and P_2) of this dark tracing band is 18 mm. on the right side of the albumin peak, 16 mm. on the left side of albumin, and 13 mm. on the left side of γ -globulin. The

light absorption shifted not only the contour edges of the pattern but also the base line which may be established by measuring the vertical distance at various points between the upper two patterns, *i. e.*, the width of the dark tracing line formed by the scanning bar. The base line, therefore, of the uppermost pattern, P_1 , is not straight but is elevated stepwise by each absorbing component from left to right. Such a corrected base line is indicated in the lower (standard) pattern. Similarly, the base line of the mid-

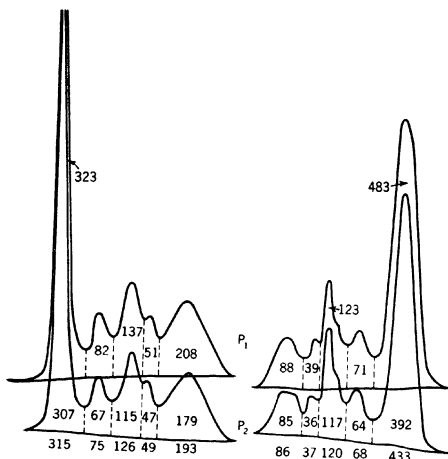


Fig. 12.—Enlarged tracings of patterns P_1 and P_2 (ascending and descending) showing the effect of light absorption by components in the cell on the position of the base line and the area of the components.¹¹

dle pattern, P_2 , slopes stepwise downward from left to right. Now that the base lines have been established the effect of light absorption on the upper contour or peaks of the patterns may be considered.

Tracings of the middle and uppermost patterns, both ascending and descending with their base lines, are shown in figure 12. The areas in planimeter units of each component are indicated. It may be observed that in the regions where the exposure is "normal" or under there is marked disagreement. Here the upper pattern is too large and the lower pattern is too small by an amount dependent on exposure. The advantage of using a double-edged scanning diaphragm was first pointed out by Svensson^{16, 17}

¹⁶ H. Svensson, *Kolloid-Z.*, **87**, 181 (1939).

¹⁷ H. Svensson, *Kolloid-Z.*, **90**, 141 (1940).

and such an arrangement has been described by Fell, Stern, and Coghill.¹⁸ The mean of the measurements made from the patterns formed by the two edges should be independent of exposure and should permit the establishment of a precise relationship between pattern area and total change in the refractive index caused by the solute at a boundary. If n_0 is the refractive index of the solvent and n_1 is the refractive index of the solution, the area, A , of the pattern resulting from a boundary is:

$$A = abmr \int_{n_0}^{n_1} (dn/dx)dx = abmr(n_1 - n_0)$$

where a is the electrophoresis cell dimension along the optic path (in cm.), b the optic distance from the middle of the cell to the scanning bar (focal plane of the Schlieren lens), m the magnification of the cell image caused by the camera lens, and r the ratio of motion of the scanning plate to the scanning bar. If the pattern is then magnified for tracing purposes this magnification factor must, of course, also be considered.

4. Modifications of Tiselius Apparatus

The original Tiselius apparatus¹⁹ is schematically illustrated in figure 13. It consists of a light source, L , the condenser lens, LC , the source slit, S ,

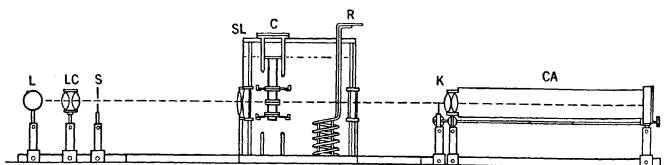


Fig. 13.—Schematic arrangement of original Tiselius apparatus.

the Schlieren lens, SL , and the electrophoresis cell, C , inside the thermostatic bath cooled by refrigerator coils, R . This apparatus gave a pattern consisting of dark Schlieren or streaks formed at the camera (CA) plate, by intercepting deviated rays with the knife edge, K . The original apparatus has been changed by several investigators. A modification by Longworth and MacInnes²⁰ has had wide usage.* The chief differences in this apparatus from the original are the adaptation of a scanning method to

* Apparatus obtainable from Klett Manufacturing Co., New York and from Frank Pearson Associates, New York.

¹⁸ N. Fell, K. G. Stern, and R. D. Coghill, *J. Immunol.*, **39**, 223 (1940).

¹⁹ A. Tiselius, *Trans. Faraday Soc.*, **33**, 524 (1937).

²⁰ L. G. Longworth and D. A. MacInnes, *Chem. Revs.*, **24**, 271 (1939)

plot boundary refractive-index gradients against their position in the cell and changes in the cell assembly so that one electrode vessel and one side of the cell are closed. Another apparatus described by Swingle²¹ uses parabolic mirrors throughout the optical system instead of lenses.

Some attempts have been made to overcome two of the principal impediments of the original Tiselius apparatus, namely, its cumbersomeness and its lack of facility in upkeep and operation. Moore and White²² have described an instrument (Fig. 14) which retains the assets of the Tiselius apparatus but is compact and simplified.* It is complete in one piece with extreme dimensions of 63 inches \times 12 inches \times 14 inches. The optics have been improved by introducing a second Schlieren lens so that parallel light traverses the cell, and by using a Longworth scanning diaphragm consisting of two edges forming a wide slit rather than a single edge. The two edges produce in effect two patterns, one atop the other on the photographic

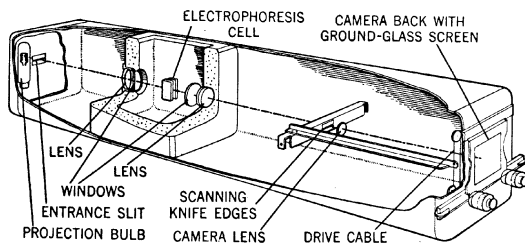


Fig. 14.—Compact electrophoresis apparatus.²²

plate, the advantages of which have been discussed (page 1700). This apparatus uses a 2-cc. Tiselius cell 50 mm. tall which has been found to be most satisfactory for general electrophoretic work. The adoption of this cell permits the aperture of the Schlieren lenses to be reduced to 2 inches instead of the usual 4 inches or more. This, in conjunction with the incorporation of a second Schlieren lens permits a fourfold reduction in optical path length without loss of quality. A temperature of 0° C. is maintained for 2-3 hours by placing 2 kg. of ice and 4 liters of water in a highly insulated plastic bath. More ice is added if necessary, the water level being self-adjusting by an overflow.

Stern has designed an electrophoresis apparatus which is built into a cabinet about the size of an ordinary home refrigerator (Fig. 15).† A varia-

²¹ S. M. Swingle, *Rev. Sci. Instruments*, 18, 128 (1947).

²² D. H. Moore and J. U. White, *Rev. Sci. Instruments*, 19, 700 (1948).

* Obtainable from *Perkin-Elmer Corp.*, Glenbrook, Conn.

† Obtainable from *American Instrument Co.*, Silver Spring, Md.

tion of the Philpot-Svensson cross-slit optical method is folded inside the cabinet and the bath is cooled by the usual refrigerator equipment.

5. Preparation of Materials

The colloid concentration for the moving-boundary method should not be too great lest boundary anomalies become appreciable. Neither should

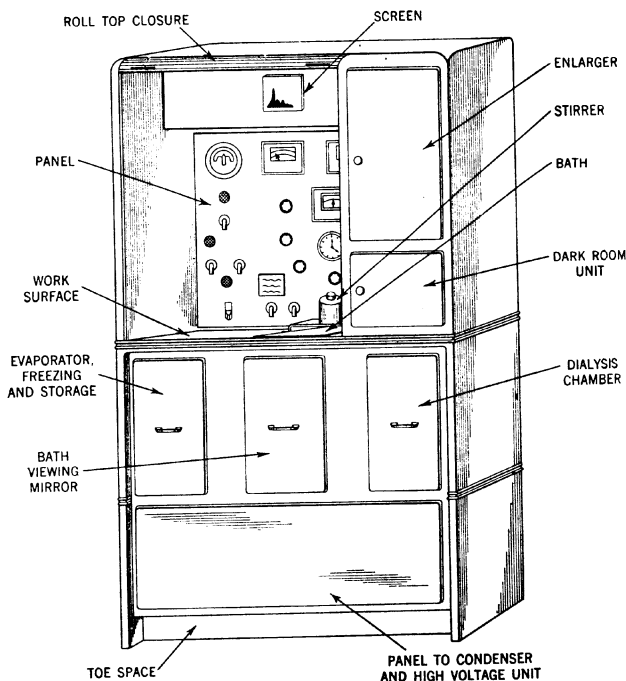


Fig. 15.—An electrophoresis apparatus designed by Stern.

it be too small lest small components become invisible. For best analytical results the concentration may range from 0.5 to 2.5%, depending upon the number of components present and the ionic strength contributed by the colloid. Colloids having high mobilities and low molecular weights contribute more to the total ionic strength and therefore must be analyzed at lower concentrations than those with low mobilities and high molecular weights. Most optical systems are capable of detecting concentrations

much lower (probably 10 times) than 0.5%, the exact value depending upon the sharpness of the boundary and its specific index of refraction increment. Higher concentrations may be employed if it is desired only to separate and recover components.

Before introduction of the material into the electrophoresis cell the species and concentration of the various ions should be as similar as possible to the buffer under which it is layered. In practice the sample is dialyzed against a buffer until Donnan equilibrium is reached. This, of course, does not produce the desired conditions if the ionic strength of the colloid is appreciable.

Dry materials may be dissolved in the buffer and used without dialysis, provided the contribution to the total ionic strength is low. In dealing with molecules too small to permit dialysis it is possible to adjust the specific conductance and pH of the colloid solution or the buffer until their values are identical by adding the proper salt, base, or acid to one of the solutions. Boundary anomalies and "false" boundaries²³ should always be suspected. If "false" boundaries are present they may be detected by their failure to vary directly with variations of total colloid concentration in different experiments,^{23, 24} but will decrease rapidly with decreasing colloid (or increasing buffer salt) concentration and usually disappear before the colloid concentration approaches zero.

The effect of dialysis on the electrophoretic pattern of human serum has been examined by the author. It was found that when serum was diluted with 2 parts of a sodium diethyl barbiturate-barbituric acid buffer (ionic strength 0.1, pH 8.6) dialysis had little effect on the pattern except for the change in colloid concentration resulting from osmosis. In a sodium phosphate-saline buffer (ionic strength 0.2, pH 7.4) the changes produced by dialysis were even less.

It has been found by the author that marked changes in the electrophoretic pattern of serum may occur when the serum is allowed to stand at room temperature even for a short time under sterile conditions. These changes occur principally in the α - and β -globulin regions and are attributed to the high lipid content of these components. Changes almost always occur in serum upon shipping unless it is refrigerated. Freezing and thawing may also cause the release of bound lipids and thus cause changes in the pattern.

Purified components such as 3 to 5 times recrystallized horse serum albumin²⁵ or egg albumin²⁶ may undergo electrophoretic changes while stored

²³ H. Svensson, *Arkiv Kemi Mineral Geol.*, **A22**, No. 10 (1946).

²⁴ D. H. Moore and J. Lynn, *J. Biol. Chem.*, **141**, 819 (1941).

²⁵ D. H. Moore and M. Mayer, *J. Biol. Chem.*, **156**, 777 (1944).

²⁶ C. F. C. MacPherson, D. H. Moore, and L. G. Longworth, *J. Biol. Chem.*, **156**, 381 (1944).

under sterile conditions in the cold. These mutations are hastened by increased temperature.

Inasmuch as hemoglobin and γ -globulin have approximately the same mobilities, hemolysis of serum or tissue extracts may cause marked changes in the apparent γ -globulin. It is impossible to judge by eye the amount of hemoglobin present, so that if there is any hemolysis the sample should either be discarded or the exact amount established by colorimetry.

6. Mobility Measurements

The mobility, μ , of a molecule is defined by the relation, $\mu = V/E$, where V is its velocity in centimeters per second when acted upon by an electric force, E , measured in volts per centimeter. The field strength in an electrophoresis cell is obtained by measuring the current, I , flowing along the cell of cross-sectional area A , the cell being filled with a medium whose conductivity is k . By Ohm's law, $E = I/kA$. Then the mobility is calculated by substituting in the equation $\mu = dkA/I$, where $d/t = V$ and $kA/I = 1/E$. The conductivity, k , is in practice measured in a conductivity

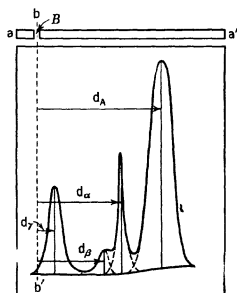


Fig. 16.—An electrophoresis pattern indicating initial boundary, B , and the way in which distances of component migration are measured.

cell which is calibrated by measuring the resistance with a Wheatstone bridge of a solution such as 0.1 N KCl whose conductivity is precisely known. If this measured resistance is different from the reciprocal value of the conductivity of the standard solution, a cell-correction factor must be used. This cell factor, K , is equal to the ratio of the measured to the calculated resistance of the standard solution. The conductivity, k , of an unknown solution measured in the calibrated conductivity cell is then $k = K/R$, where R is the measured resistance. For calibrating a conductivity cell the following example is given: the conductivity, k , of 0.1 N KCl at 0° C. is 0.00714 reciprocal ohm. If this solution when placed in an

uncalibrated conductivity cell has a resistance, R , of 152 ohms at 0°C., the cell constant, K , is determined by (see Chapter XXV):

$$K = 152 \left/ \frac{1}{0.00714} \right. = 1.086$$

The distance, d , in the moving-boundary method is measured on the photographic plate as is illustrated in the diagram of figure 16, where aa' is a photograph of the cell after the boundary has been shifted from underneath the flanged plates of the cell but before the current is switched on. The boundary is indicated by the gap. Horizontal distances from the midpoint of this gap (line bb') to a line dividing in half the area of peaks representing each component give the values of d to be substituted in the mobility formula which may now be written as follows:

$$\mu = dKA/IRtm$$

where m is the magnification factor of the electrophoresis camera. If lateral displacement of the plate has occurred between successive exposures, it is necessary to correct d for this displacement. The displacement is determined by observing whether the ends of the line containing the starting point coincide with the vertical edges of the pattern.

As is indicated in the following section on interpretation of patterns, the conductivity of the solution is not invariant throughout the cell, so that in order to obtain precise mobilities the conductivity at each boundary should be known. Whenever this is impossible best results may be obtained by averaging ascending and descending values.

7. Interpretation of Patterns

Inasmuch as the Tiselius cell makes it possible to obtain an ascending and descending pattern, one of the first guiding landmarks to be pointed out is the symmetry of these patterns. Only under the most ideal conditions will the ascending pattern be an exact mirror image of the descending pattern and in order to interpret the meaning of either one the cause of the asymmetry, first explained by Longworth and MacInnes²⁷, should be understood. In figure 17 an example of complete lack of symmetry between ascending and descending pattern is illustrated. This pattern represents an electrophoretic analysis of a 2.4% solution of cattle globin in a glycine-HCl buffer of ionic strength 0.1 at pH 2.50 after a dialysis of the globin solution against the buffer for a period of three days at which time the specific conductance of the buffer was 0.00616 and that of the protein solution was 0.00615. Samples at different levels were removed from the

²⁷ L. G. Longworth and D. A. MacInnes, *J. Am. Chem. Soc.*, **62**, 705 (1940).

cell after electrophoresis and the conductivity and *pH* of each sample were measured and were found to be different, as indicated in figure 17. These differences exist because after dialysis there is a difference in salt ion composition of the protein solution and its dialysate and because the protein ions contribute measurably to the ionic strength of the protein solution but nothing to the buffer above it in the cell.

Although the total area of the ascending pattern is equal to that of the descending pattern, the number and shape of the boundaries are very different. In the ascending pattern the fastest boundary, *F*, is sharper than would have occurred as a result of diffusion. This abnormal sharpening is a result of the difference in the specific conductance of the fluid above and

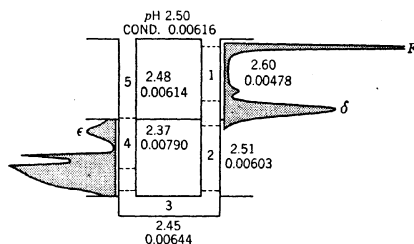


Fig. 17.—Ascending and descending patterns after electrophoresis of a 2.4% solution of beef globin in glycine-HCl buffer of ionic strength 0.1. Values of *pH* and conductivity after electrophoresis are indicated.

below this boundary. Since the current density throughout the cell is uniform, the voltage gradient at any point in the cell is, by Ohm's law, inversely proportional to the conductivity. Therefore the molecules which tend to lag behind either by diffusion or by electrophoretic heterogeneity (there are actually two components in this boundary) find themselves in a stronger field and are accelerated, whereas those which tend to go ahead find themselves in a weaker field and are decelerated. The opposite effect exists at the fastest descending boundary whose pattern indicates the presence of two or more components.

There is a large almost immobile boundary on the ascending side. This boundary may be due entirely to a salt gradient and a superimposed protein gradient resulting from the difference in conductivity on the two sides of this boundary. As protein ions of the faster components cross this δ boundary they enter a stronger electric field, are accelerated, and move with a higher velocity than they did before reaching this point. Hence, since the protein ions move with a greater velocity in the regions above the δ boundary their concentration is proportionally less.

On the descending side the ϵ boundary is probably due entirely to the refractive increment of the buffer salts. The asymmetry just described and the boundary effects are, as shown by Longworth and MacInnes²⁸ and by Svensson,²⁹ a function of the ratio of protein ion to buffer ion concentration. If a 1% globin solution is analyzed in the same buffer the patterns illustrated in figure 18 are obtained. These ascending and descending patterns have a greater resemblance to each other and the δ and ϵ boundaries are much smaller but there is still lack of symmetry between the two patterns. As the protein concentration is further reduced and made to approach zero, the ascending and descending patterns become mirror images of each other and the δ and ϵ boundaries disappear.

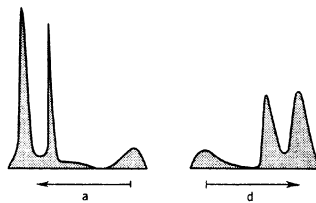


Fig. 18.—Same as Figure 17 except that the concentration of globin was reduced to 1%.

Besides the shift in total salt ions, as indicated by conductivity, there is also a shift in the hydrogen-ion concentration, as indicated by pH measurements. Thus the mobility and shape of any boundary are affected by both the changes in conductivity and pH across the boundary. These two factors may augment or oppose each other. In the patterns illustrated in figure 17 the pH change across the fastest ascending boundary, F , has an opposite effect to that of the conductivity because the mobilities of these proteins decrease as the pH is raised. The change in conductance here, however, has the predominating influence. The complete theory of multiple moving boundaries has not yet been developed, although much progress has been made recently.³⁰⁻³⁴

In order to convert pattern areas into colloid concentration two factors

²⁸ L. G. Longworth and D. A. MacInnes, *J. Am. Chem. Soc.*, **62**, 705 (1940).

²⁹ H. Svensson, *Arkiv Kemi Mineral. Geol.*, **A17**, No. 14 (1943).

³⁰ S. H. Armstrong, Jr., M. J. E. Budka, and K. C. Morrison, *J. Am. Chem. Soc.*, **69**, 416 (1947).

³¹ L. G. Longworth and D. A. MacInnes, *Chem. Revs.*, **24**, 271 (1939).

³² L. G. Longworth and D. A. MacInnes, *J. Am. Chem. Soc.*, **62**, 705 (1940).

³³ V. Dole, *J. Am. Chem. Soc.*, **67**, 1119 (1945).

³⁴ H. Svensson, *Arkiv Kemi Mineral. Geol.*, **22A**, No. 10 (1946).

must be considered: (1) the specific refraction increment of each component; and (2) the contribution of buffer salt gradients and of the other colloidal components to the total or measured refractive increment of each boundary.

Biological products do not usually consist of substances having identical specific indices of refraction. Furthermore, an electrophoretically homogeneous component may not necessarily represent a single substance but one or more entities with the same mobility yet having different specific refractive increments. For example, the component of highest mobility in serum is usually designated as albumin and it may be a single substance

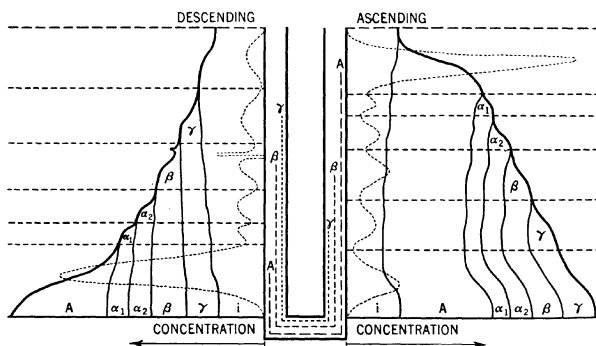


Fig. 19.—Concentration diagram of serum components after electrophoresis: *i* represents the index of refraction increment of the buffer salts.

with a characteristic specific refraction increment. However, it may represent a composite or complex of several substances such as glycoproteins or lipoproteins with the same mobility but all having different specific indices of refraction, only the average of which can be measured unless they are separated by other means.

The area of a component in a pattern could not be converted into absolute concentration even if the specific refraction increment of the component were known because, as is qualitatively indicated in figure 19, the concentration of each component in the system is affected by the presence of each other component. In figure 19 an electrophoresis cell is schematically illustrated. On each side of the cell an electrophoresis pattern of human serum obtained in diethyl barbiturate buffer is indicated by the dotted lines. This curve plots rate of change of refractivity against position in the cell. The heavy lines plot the actual concentration against position in the cell. The approximate positions and extent of three components, albumin and β - and γ -globulins, are indicated by the broken lines inside the cell.

In the ascending limb the concentration of albumin rises from zero at the top of the cell to a value indicated by the area under the gradient curve. At the various globulin boundaries there is an unknown decrease in albumin concentration. Moreover, there are decreases in buffer salt concentration at each boundary (see Fig. 17) and at the δ boundary there is an increase with distance down the cell of all the serum fractions as well as the buffer. Similarly the concentration curves on the descending limb indicate the kind of concentration changes that take place at each boundary there.

VI. APPLICATIONS

It is not intended to give in this chapter a review of the literature on the applications of electrophoresis. Such a review has recently been made by Luetscher³⁵ and many other monographs and review articles have appeared during the past few years.³⁶⁻³⁸ Some of the most typical examples in various fields of application will, however, be mentioned.

The immediate reason for Tiselius' development of the improved electrophoresis apparatus was to study blood serum. The history of the analysis of serum contains numerous attempts to fractionate it into constituent proteins ever since serum albumin was crystallized by Guerber. Although serum could be separated into several fractions with different solubilities, these fractions did not fulfill the criteria required to establish them as chemical individuals. Thus Sørensen conceived of serum as a system composed of reversibly dissociable components. Indeed, it was felt that many fractions isolated from serum may have been the results of the treatment of the serum during their isolation. Although the ultracentrifuge (see Chapter XIII) gave much valuable information regarding the nature of serum and its protein in normal and pathologic conditions, it remained for Tiselius to provide a simple means of attack capable of removing the fractions after their separation. Tiselius found that human serum untreated with precipitating salts, when placed in an electric field, begins to separate into four components. The fastest of these components was identified as albumin and the slower three components as globulins, and were named α , β , and γ . In plasma the fibrinogen appeared between the β - and γ -globulins. It has been found that sera from a number of species have similar yet characteristically different electrophoresis patterns.^{39, 40} When isolated by electrophoresis and re-examined, the separate constituents of serum exhibit electrical mobilities essentially the same as those found in the unseparated serum. This is important evidence that the individual constituents are present

³⁵ J. A. Luetscher, Jr., *Physiol. Revs.*, **27**, 621 (1947).

³⁶ H. A. Abramson, L. S. Moyer, and M. H. Gorin, *Electrophoresis of Proteins and the Chemistry of Cell Surfaces*. Reinhold, New York, 1942.

³⁷ D. A. MacInnes and L. G. Longworth, *Colloid Chemistry Theory and Methods*. Vol. V, Reinhold, New York, 1944, p. 387.

³⁸ K. G. Stern and M. Reiner, *Yale J. Biol. Med.*, **19**, 67 (1946).

³⁹ D. H. Moore, *J. Biol. Chem.*, **161**, 21 (1945).

⁴⁰ H. F. Deutsch and M. B. Goodloe, *J. Biol. Chem.*, **161**, 1 (1945).

in the serum and does not support the view that serum is a continuous mixture. The effect of buffer solvents on electrophoretic patterns cannot be overemphasized, however, because the number, relative size, and mobility of components, and the contour and symmetry of ascending and descending patterns are all a function of the electrolyte used.

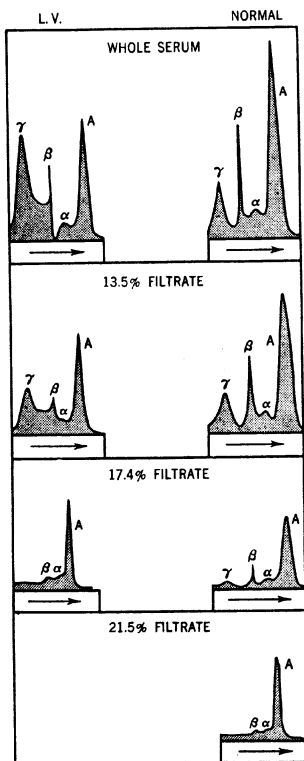


Fig. 20.—Electrophoresis patterns in hyperglobulinemia due to lymphogranuloma venereum and in normal human serum.⁴¹ Diminution of patterns with increasing salt concentration is due to dilution. Patterns are of whole serum and of filtrates after fractional precipitation with sodium sulfate (Howe method).

An electrophoretic analysis of the fractions obtained by the Howe method from a pathological and a normal serum is indicated in figure 20. The 13.5% sodium sulfate filtrate represents the fraction after the euglobin has been removed, the 17.4% filtrate after the pseudoglobulin I has been removed, the 21.5% filtrate after everything except the albumin is supposedly removed. A considerable amount of α - and β -globulins remains with the albumin. Similar results have been found by Cohn

⁴¹ A. B. Gutman, D. A. Moore, E. B. Gutman, V. McClellan, and E. A. Kabat, *J. Clin. Invest.*, 20, 765 (1941).

*et al.*⁴² using different salts. On averaging A/G ratios from 25 normal sera, Moore and Lynn⁴³ have found that the electrophoretic A/G ratio has a value of approximately 2, considerably less than that given by the Howe method in 36 cases, according to Gutman *et al.*⁴⁴ This was to be expected because not all of the globulins are precipitated by 21.5% saturated sodium sulfate.

In the study of sera more information may be obtained by measuring not only pattern areas for each component but also in determining the nature of the substance causing the refractive-index gradients. Since the colloid of serum is a composite of protein, lipid, and carbohydrate, each of which may vary, much information may be acquired by determining the variation of each of these constituents. The increased lipid content in serum in such diseases as obstructive jaundice and nephrosis has been demonstrated by Longworth.^{45, 46} McFarlane⁴⁷ and Zeldis *et al.*⁴⁸ have examined the lipids in human and dog sera under various conditions and have shown the decrease in the ratio of pattern area to nitrogen after the sera were extracted with lipid solvents. Changes in the pattern of human sera during pregnancy and the increase of α - and β -globulins in infants during the first few days of life⁴⁹ and the sex differences in fowl serum⁵⁰ have been shown to result largely from lipids. The variation of carbohydrates in serum has not been extensively studied but the few available data indicate a fruitful field.⁵¹⁻⁵⁴ The electrophoretic method in conjunction with other identifying procedures offers an excellent means of study because it is sensitive to all substances, whereas other methods are frequently sensitive to only specific substances.

Electrophoresis probably serves its greatest utility in the field of organic chemistry as an aid in the purification of proteins such as antibodies, enzymes, hormones, viruses, etc. In the study of antibodies where the concentration is large enough to be observed in the pattern its location and concentration may be noted by comparing patterns taken before and after absorption with the specific antigen. This was the case in the hyperimmune horse and rabbit sera studied by Tiselius and Kabat⁵⁵

⁴² E. J. Cohn, *Chem. Revs.*, **28**, 395 (1941).

⁴³ D. H. Moore and J. Lynn, *J. Biol. Chem.*, **141**, 819 (1941).

⁴⁴ A. B. Gutman, D. A. Moore, E. B. Gutman, V. McClellan, and E. A. Kabat, *J. Clin. Invest.*, **20**, 765 (1941).

⁴⁵ L. G. Longworth, T. Shedlovsky, and D. A. MacInnes, *J. Exptl. Med.*, **70**, 399 (1939).

⁴⁶ L. G. Longworth and D. A. MacInnes, *J. Exptl. Med.*, **71**, 77 (1940).

⁴⁷ A. S. McFarlane, *Nature*, **149**, 439 (1942).

⁴⁸ L. J. Zeldis, E. L. Alling, A. B. McCoord, and J. P. Kulka, *J. Exptl. Med.*, **82**, 411 (1945).

⁴⁹ D. H. Moore, R. Martin Du Pan, and C. L. Buxton, *Am. J. Obstet. Gynecol.*, **57**, 312 (1949).

⁵⁰ D. H. Moore, *Endocrinology*, **42**, 38 (1948).

⁵¹ T. L. McMeekin, *J. Am. Chem. Soc.*, **62**, 3393 (1940).

⁵² D. H. Moore, S. C. Shen, and C. S. Alexander, *Proc. Soc. Exptl. Biol. Med.*, **58**, 307 (1945).

⁵³ D. H. Moore, L. Levin, and G. K. Smelser, *J. Biol. Chem.*, **157**, 723 (1945).

⁵⁴ F. B. Seibert, M. V. Seibert, A. J. Atno, and H. W. Campbell, *J. Clin. Invest.*, **26**, 90 (1947).

⁵⁵ A. Tiselius and E. A. Kabat, *J. Exptl. Med.*, **69**, 119 (1939).

and others.^{56, 57} In most instances the concentration of antibody, however, is below the threshold of sensitivity of the apparatus. It is then necessary to resort to assaying separated samples (see page 1694) by techniques such as complement fixation, agglutination, neutralization, etc. Several examples of this procedure are in the literature.⁵⁸⁻⁶⁰

In the procedure of isolating and purifying enzymes, hormones, and viruses,⁶¹⁻⁶⁸ electrophoresis serves as a most useful tool because it is such a gentle process it does not destroy activity and recovered fractions usually lend themselves to assay.

⁵⁶ D. H. Moore, J. van der Scheer, and R. W. G. Wyckoff, *J. Immunol.*, **38**, 221 (1940).

⁵⁷ J. van der Scheer, R. W. G. Wyckoff, and F. H. Clarke, *J. Immunol.*, **39**, 65 (1940).

⁵⁸ N. Fell, K. G. Stern, and R. D. Coghill, *J. Immunol.*, **39**, 223 (1940).

⁵⁹ B. Davis, D. H. Moore, E. A. Kabat, and A. Harris, *J. Immunol.*, **50**, 1 (1945).

⁶⁰ H. Koprowski, G. Richmond, and D. H. Moore, *J. Exptl. Med.*, **85**, 515 (1947).

⁶¹ D. Glick, S. Glaubach, and D. H. Moore, *J. Biol. Chem.*, **144**, 525 (1942).

⁶² V. Ross, D. H. Moore, and E. G. Miller, Jr., *J. Biol. Chem.*, **144**, 667 (1942).

⁶³ D. H. Moore, *J. Biol. Chem.*, **161**, 597 (1945).

⁶⁴ A. Tiselius, G. E. Henschen, and H. Svensson, *Biochem. J.*, **32**, 1814 (1938).

⁶⁵ C. H. Li, W. R. Lyons, and H. M. Evans, *J. Gen. Physiol.*, **23**, 433 (1940).

⁶⁶ R. A. Shipley, K. G. Stern, and A. White, *J. Exptl. Med.*, **69**, 785 (1939).

⁶⁷ T. Shedlovsky and J. E. Smadel, *J. Exptl. Med.*, **72**, 511 (1940).

⁶⁸ D. G. Sharp, A. R. Taylor, D. W. Beard, and J. W. Beard, *J. Biol. Chem.*, **142**, 193 (1942).

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POTENTIOMETRY

L. MICHAELIS

The Laboratories of The Rockefeller Institute for Medical Research

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I. INTRODUCTION. NORMAL HYDROGEN ELECTRODE AND LIQUID JUNCTION POTENTIAL

Potentiometry consists of the measurement of the electromotive force of a galvanic cell composed of two half-cells one of which is a reference half-cell of known composition, and the other an electrode immersed in the solution to be investigated. It is customary to define as the zero point of the potential that of a hydrogen electrode at 1 atmosphere hydrogen-gas pressure immersed in a solution at $pH = 0$, and held at the same temperature as the other half-cell. The methods dealt with in this article do not take into consideration potential differences of two half-cells with different temperatures.

The standard electrode just defined is not suitable for practical use because a solution with $pH = 0$ establishes a considerable liquid junction potential in contact with another solution. This happens not only on direct contact but even if a saturated solution of potassium chloride is interposed, although this method is in general most efficient in minimizing junction potentials.¹ It is obvious that, in the presence of a junction potential, the electromotive force of the cell is not identical with the potential difference of the two half-cells. Only in special cases which rarely materialize can the junction potential be calculated with some accuracy. In general, it can neither be calculated nor accurately measured and it is usually not even constant in time as diffusion progresses.

In practice, the reference electrode is usually a calomel electrode saturated with potassium chloride. Because of the approximate, although not complete, equality of the mobility of K^+ and Cl^- ions, a saturated KCl solution develops only a very small, allegedly negligible, liquid junction potential with any other aqueous solution, provided the latter is not extremely acid or alkaline, and its ionic strength is low (say, <0.2). But even in the best cases, the junction potential is only approximately eliminated and whatever remains of it cannot be measured or calculated exactly. This uncertainty would be irrelevant if the junction potential were the same under all practically occurring conditions. However, this is not the case, and the possibility of the measurement of any electrode potential therefore depends on the following premises: The liquid junction potential of the cell must be negligibly small, *i. e.*, although it may vary according to the nature of the solutions, it must have only a negligible effect on conclusions which are to be derived from the measured *e. m. f.* of

¹ Potentiometric methods involving cells without liquid junction are of restricted use, from a practical, methodological point of view, although they are very important from theoretical aspects, as discussed by H. S. Harned and R. W. Ehlers, *J. Am. Chem. Soc.*, **54**, 1350 (1932), and D. I. Hitchcock and A. C. Taylor, *ibid.*, **59**, 1812 (1937).

the cell. For example, if the pH of two different solutions is measured with the hydrogen electrode and, in both cases, the liquid junction potentials are identical, the pH difference of the two solutions can be determined precisely according to the theory. However, if the liquid junction potentials in both cases may differ by up to 1 mv., the pH measurement will be uncertain by up to 0.018 pH unit. An uncertainty with respect to 5 mv. involves an uncertainty in pH of 0.09 unit. The organic chemist uses potential measurements mainly to calculate values of pH and of oxidation-reduction potentials. Strictly speaking, even the measurement of pH with the hydrogen electrode is the measurement of an oxidation-reduction potential, the redox system consisting of hydrogen and hydrogen ion.

II. POTENTIOMETER

The fundamental method of measuring the electromotive force of a galvanic cell is the "compensation method," represented by figure 1. The cell to be measured, C , is placed in what may be called a shunt, $A-C-G-F$, in

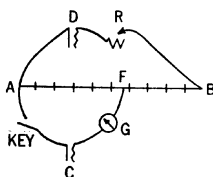


Fig. 1.—Schematic diagram of potentiometer.

the circuit $A-D-R-B-F-A$, where D is a galvanic cell of large capacity and of an electromotive force which keeps constant during a long time so as not to vary during the experiment. At point R there is interposed in the circuit an adjustable resistance. AB is in this scheme a straight wire of uniform cross section, stretched over a scale with marked divisions. The resistance, R , is set so that each line of division corresponds to a certain potential gradient, say 1 mv. per mm. The unknown cell, C , is shunted to this graduated wire between the fixed point, A , and the gliding contact, F . In order to measure the e. m. f. of C , this gliding contact is shifted so that the galvanometer, G , shows no current. Then, the e. m. f. of cell C equals the potential difference between A and F , which is read at the scale: The current arising from cell C is compensated by that fraction of the current arising from D which passes, in opposite direction, through shunt $A-C-G-F$.

In practice, wire $A-B$, with its millimeter scale, will be replaced by a

series of resistance coils, in series with an additional, adjustable sliding resistance. What is essential is that the resistance from *A* to *B* remains constant all the time, and that by some means this resistance can be divided into two parts. In the commercially available potentiometers, there is usually a discontinuous adjustment in terms of 100 mv. each (provided the resistance, *R*, is properly adjusted), and in series with it a sliding resistance corresponding to, say, 1 mv. for each line on the scale. The scale should make possible readings to 0.1 mv. For most purposes "student potentiometers" will be sufficient. They allow direct readings to one millivolt and an estimation of the tenths of a millivolt. More elaborate potentiometers allow accurate readings down to one-twentieth of a millivolt, and, by a suitably arranged shunt, even down to one two-hundredth of a millivolt. The latter is unnecessary in most of the experimental work with galvanic cells of this type. Many types of commercial potentiometers are available, all operating on the principle described above.

The correct setting of the resistance, *R*, is carried out by inserting in *C*, instead of the unknown cell, a *standard cell of known e. m. f.* This is usually a Weston cell, built with cadmium amalgam in cadmium sulfate solution, and in the other half, mercury in the same solution of cadmium sulfate saturated with mercurous sulfate. There are two types of this cell, one using saturated cadmium sulfate with excess of solid salt, the other using a solution of cadmium sulfate saturated at 4° C. without the solid salt. The latter is the commercial type of standard cell and has the advantage of having a negligible temperature coefficient of the e. m. f. and of establishing the final potential immediately on changing the temperature. Its e. m. f. should be checked about once a year. It amounts usually to 1.0817 v. Never should any current be withdrawn from the cell amounting to more than a small fraction of one milliampere. The cell must never be short-circuited and should be used only when inserted in the potentiometer circuit which has a sufficiently high resistance. If, by accident, the cell has been short-circuited, one should give it a few days to recover its original e. m. f.

The cell, D, which furnishes the permanent current, is represented either by a two-volt large-capacity storage cell, or by another cell of fairly constant e. m. f. and high capacity, such as the "air cell" ("Everready"), or by some good dry cell of which a few may be used in parallel to increase the capacity. If a storage cell is used, its voltage should never fall below 1.98 v., and the circuit as indicated in figure 1 should be closed for about ten minutes before the beginning of the experiment to establish a constant e. m. f. With a freshly recharged storage cell, it may take longer to establish a constant e. m. f. In commercial potentiometers, the switching from the circuit with the standard cell to that with the unknown cell is usu-

ally performed in a more convenient way than described here; instructions will be found with each model.

The key represented in figure 1 within shunt $A-C-G-F$ should be closed as briefly as possible as long as the adjustment of the variable contact at F has not yet established nearly complete compensation of the current. Only when the galvanometer shows almost no deflection on closing this switch, may it be closed for a somewhat longer time to facilitate the final reading. Closing of the circuit in an uncompensated condition for an appreciable time may polarize the cell to be measured, unless its capacity is very high, and change its e. m. f., making it necessary to wait for its recovery. Just a little attention will eliminate erroneous results due to this possibility in cells of the type used here. This source of error will be practically important only for cells of a very low capacity, *e. g.*, in measuring pH in extremely poorly buffered solutions, or in measuring the oxidation-reduction potential of very dilute redox systems especially if they contain the oxidized and the reduced forms in an extreme ratio ($> 10:1$ or $< 1:10$, respectively).

Usually potentiometers have several (three) switches instead of one, enclosing in the circuit various resistances or shunts. As long as the compensation is far from being complete, the one with the highest resistance is used to avoid both polarization and unduly large deflections of the galvanometer needle. The switch introducing no accessory resistance is used only when the compensation is nearly reached. The galvanometer (G in Fig. 1) should be sensitive enough, but not more than necessary. There are few cases of cells containing electrolytes in a concentration of, say, $10^{-2} M$ or lower, where the lamp and scale enclosed galvanometer (*Leeds & Northrup*) will fail to function. For finer work, a galvanometer of higher sensitivity (10^{-8} amp. per mm.) can be used, with a short period of deflection (a few seconds). The galvanometer must be critically damped so that after breaking the circuit the pointer (light beam from the movable mirror) swings back to resting position without oscillation. This condition is effected by shunting the galvanometer through a suitable resistance. In many of the commercial potentiometers the critical damping requires no further attention. Otherwise one has to find a shunt of suitable resistance, which may vary widely, according to the type of potentiometer, depending upon the inner resistance of the galvanometer.

Whenever the resistance of the cell to be measured is very high, *e. g.*, in working with the glass electrode, where it may range from one million to one hundred million ohms, the simple potentiometer outlined above fails to function. In this case, a quadrant electrometer may be used instead of a galvanometer. Although this method is excellent, probably the most satisfactory of all, it will not be discussed here because of the intricacy of

the necessary equipment. Moreover, for all practical purposes it may be considered as superseded by the potentiometers with electronic tubes.

The use of electronic tubes has been developed to a wide extent in recent years and is now generally adopted for pH measurement with the glass electrode. Since the earlier development of such instruments for use in chemical laboratories² many types of electronic-tube potentiometers have been manufactured by various firms, using various devices, including accessory equipment such as shielding devices, ready-made calomel cells, and glass electrodes. A general description of these instruments is not

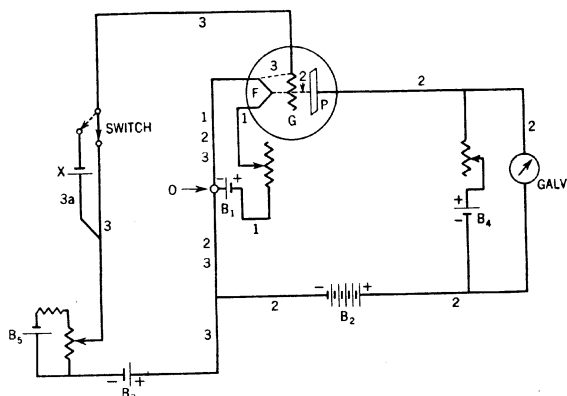


Fig. 2.—Schematic diagram of electronic-tube potentiometer.

possible in this chapter, not only because of their complexity, but even more so because the different models vary widely and the details of their construction have not been published. Dole's³ monograph will serve as a good source of as much detailed information as is available. The fundamental principle on which those instruments depend may be explained in the following manner.

Point *O* (Fig. 2) is common for the three circuits, 1, 2, and 3, produced by the three batteries, B_1 , B_2 , and B_3 . The potential at this point may be taken as the reference, or zero, potential of the system. Circuit 1 heats the filament of the electronic tube to a temperature high enough to cause emission of electrons. Those electrons are attracted by the plate, *P*, which is strongly positively charged by the battery B_2 ; this battery produces circuit 2. A part of this circuit is represented by the electrons moving from the filament across the vacuum toward the plate. Bat-

² See, for example, A. B. Hastings and J. Sendroy, *J. Biol. Chem.*, **61**, 695 (1924).

³ M. Dole, *The Glass Electrode*. Wiley, New York, 1941.

tery B_2 establishes the potential of the grid which in the ideal case controls the flow of the electrons from the filament to the plate by its electrostatic field. Battery B_3 has the purpose of imparting a definite, properly chosen potential to the grid so that it will receive no electrons from the filament or, in the case of a nonideal vacuum tube, receive an equal number of positive (from positive ions) and negative charges and therefore draw no current from the cell to be measured.

Plate current \mathcal{I} passes through a galvanometer. Another battery, B_4 , produces a current in the opposite direction and is regulated by a variable resistance so as to compensate the plate current and bring the galvanometer to some zero position. Whenever the grid charge is altered, the intensity of the plate current is also altered and the galvanometer is deflected. The unknown battery, X , containing the glass electrode, is now switched into circuit 3 (circuit 3a). Its electromotive force will cause a change of the grid potential and deflect the galvanometer. A potentiometer run by battery B_5 is used to oppose the potential of the unknown battery, X , and bring the grid back to the point of zero grid current. The reading of the potentiometer then indicates the e. m. f. of the unknown cell.

Calomel Electrode as Reference

It has been explained why the "normal hydrogen electrode" is, in practice, no adequate reference electrode, although, by definition, the potential of an idealized normal hydrogen electrode has been chosen as the zero point of potential. A much better reference electrode for practical use is the calomel electrode, which of course does not have zero potential but must be calibrated with respect to the idealized normal hydrogen electrode. It consists of mercury in contact with a saturated solution of calomel in a potassium chloride solution. According to the concentration of potassium chloride, one distinguishes between the 0.1 N , the 1 N , the 3.5 N , and the saturated calomel electrode. The 3.5 N electrode has the advantage that with varying temperature its potential is established immediately, while the saturated electrode may show a slight lag.⁴ The writer advises the use of the 3.5 N electrode if it is kept in a room of varying temperature, and of the saturated electrode if it is kept at constant temperature. It is easier to establish a set of calomel electrodes with strictly equal, reproducible potentials with the 0.1 N than with the saturated (or 3.5 N) electrode. However, the latter has, among other features, the advantage that its potential difference against the hydrogen electrode with standard acetate, which represents the most practicable reference value for all measurements, varies little with temperature. This statement holds, of course, only when the calomel electrode and the acetate electrode have the same temperature.

⁴ D. A. MacInnes, D. Belcher, and T. Shedlovsky, *J. Am. Chem. Soc.*, **60**, 1094 (1938).

Pure mercury which is free from all other metals is available commercially for calomel electrodes. This purity can be obtained by distilling twice a good grade of mercury, once with air bubbling through to oxidize baser metals, and then *in vacuo*. Another purification may be applied by shaking the mercury for several minutes with dilute nitric acid (10%) containing a small amount (1%) of mercurous nitrate. For further details see Chapter XXVIII (Polarography) by Müller.

To obtain good *calomel*, complicated prescriptions have been advocated. However, satisfactory brands of "calomel for calomel electrodes" are available commercially. If it is not yet mixed with mercury, the calomel powder should be ground in a mortar with small amounts of pure mercury and saturated potassium chloride solution, and the gray paste obtained used for the electrode. The mercury reduces any trace of mercuric chloride present in mercurous chloride. If the *potassium chloride* is not guaranteed to be pure, it should be recrystallized a few times, to insure that the concentration of its saturated solution in the presence of solid potassium chloride is independent of the amount of solid crystals.

A suitable form of the vessel is that of figure 3. A short platinum wire is fused into its lowest point, thus establishing a metallic contact across the glass septum to tube *B*. The outlet, *C*, should contain a glass stopper or at least terminate in a rubber tube with clamp. The vessel is filled with mercury up to mark *a*. Care must be taken that the vessel is dry and that the platinum wire never becomes wet during the following operations. Great

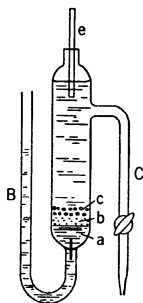


Fig. 3.—Calomel electrode.

trouble with respect to lack of constancy in potential may be encountered if this precaution is not observed. The calomel paste is layered upon the mercury up to mark *b*, and, to make a KCl-saturated electrode, solid, finely powdered potassium chloride up to mark *c*. Then saturated potassium chloride solution is sucked into almost the top of the vessel and the stopcock (or the clamp) at the outlet is closed. Glass tube *e* is now replaced by a thermometer. The outlet is then immersed under the surface of a saturated potassium chloride solution in a beaker. The surface of this solution is covered with a film of white mineral oil, 2 mm. thick, to prevent evaporation of water and the formation of crusts of solid potassium chloride. The tip of the electrode should never be lifted out of this solution, which scarcely ever needs

renewal. The electrode should be carefully kept at rest and never shaken, or the contact between the platinum tip and the mercury may become wet, causing irreparable trouble with respect to the constancy of the potential. It may take some days before the potential reaches its definite value; even so, the potential should be checked against the hydrogen electrode with standard acetate from time to time. It is not necessary that the value obtained agree precisely with any standard value recorded in the

literature, but it should neither drift nor vary in an irregular manner. This tolerance is possible because the value is used only as a reference in the comparison of a standard buffer with the unknown solution.^{4, 5}

A calomel electrode very convenient for transportation can be obtained by filling it with a KCl-saturated agar gel instead of with an aqueous potassium chloride solution. This is made by boiling 3 g. of agar, 40 g. of potassium chloride, and 100 ml. of water to complete solution. It is filled in while hot and then allowed to cool for solidification. No stopcock at the outlet, or rubber tubing with clamp, is needed. There is no guarantee that the potential of such an electrode is quite the same as that of the other, but after some aging it will be constant in time.

III. DETERMINATION OF pH^6

1. Hydrogen Electrode

The hydrogen electrode consists of a platinum electrode coated with platinum black, in a vessel which allows bubbling through of hydrogen gas (Fig. 4). The electrode is made of a small plate of platinum (2×3 mm.)

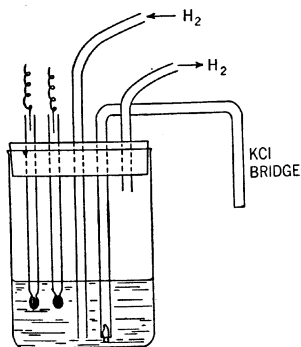


Fig. 4.—Simple hydrogen electrode vessel.

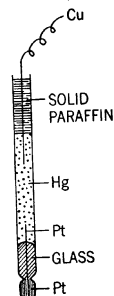


Fig. 5.—Platinum electrode.

⁵ D. I. Hitchcock and A. C. Taylor, *J. Am. Chem. Soc.*, **59**, 1812 (1937); **60**, 2710 (1938).

⁶ S. P. L. Sørensen, *Compt. rend. trav. lab. Carlsberg*, **8**, 1, 39 (1909). L. Michaelis, *Die Wasserstoffionen-Konzentration*, Springer, Berlin, 1914; 2nd ed., Berlin, 1922. English translation of 2nd ed. by W. A. Perlzweig, Williams & Wilkins, Baltimore, 1926. W. M. Clark, *The Determination of Hydrogen Ions*, Williams & Wilkins, Baltimore, 1920; 3rd ed., 1928. I. M. Kolthoff and N. H. Furman, *Potentiometric Titration*, Wiley, New York, 1931. D. A. MacInnes, *The Principles of Electrochemistry*, Reinhold, New York, 1939.

welded to a Pt wire (Fig. 5). It is even sufficient to use the protruding end of the platinum wire (about 5 mm. long) without attaching the plate, although the higher capacity of an electrode with larger surface is advantageous. The wire is melted into a glass tube in such a way that it is entirely surrounded to a length of at least 5 mm. by glass. The glass should have a thermal expansion coefficient as equal as possible to that of platinum, to avoid any leak between the wire and the glass. The interior of the glass tube is filled with mercury, a copper wire is inserted, and the top is sealed with paraffin. It is recommended that two such electrodes be used in one vessel. They should show precisely the same potential. Figure 4 illustrates a simple half-cell utilizing the platinum electrode. A cylindrical glass vessel is closed with a well-fitting rubber stopper with several holes, one for each electrode, one as an inlet for hydrogen, another as an outlet, and one for the potassium chloride bridge. It is advisable to have an additional hole for a thermometer. The hydrogen gas is most conveniently obtained from a commercial cylinder. Experience shows that although the gas may not be strictly pure it is pure enough so that further purification will not change the results. If the hydrogen is generated from zinc and sulfuric acid in a Kipp gas generator, it must be purified by first bubbling it through a gas wash bottle containing a solution of potassium permanganate and another containing 10% potassium hydroxide (not sodium hydroxide, because sodium carbonate is insoluble in sodium hydroxide and may clog the tubes). Tank hydrogen may be purified by passing it over red-hot platinum wire to burn up any trace of oxygen or preferably by the method described in the section on redox potentials (page 1764).

A. PLATINIZING A PLATINUM ELECTRODE

The following procedure for depositing platinum black at a blank platinum electrode is recommended. The platinum electrode is used as the cathode in 5 to 10 ml. of a 3% solution of "platinum chloride" (chloroplatinic acid), in a small beaker, *e. g.*, a weighing vessel. Another blank platinum electrode is used as anode. A current just strong enough, *e. g.*, from a four-volt battery, with appropriate resistance, is used to bring about not too sluggish a development of gas at the electrodes. Addition of a trace (0.1 mg.) of lead acetate to the solution speeds up the deposition of platinum black. Although this addition has been assumed to produce erroneous results by some authors, the writer has never encountered any difficulties. The surface of the electrode must be thoroughly cleaned in concentrated sulfuric acid and by repeated thorough washing in distilled water, before the platinization is started. The deposit of black should be uniform over the whole surface of the electrode. The process should be continued no longer than just to give a very thin black coat. Too thick a coating may be harmful insofar as it may require more time to attain equilibrium with hydrogen when

later used in a pH measurement. After the surface is uniformly covered, the electrode is washed repeatedly with distilled water and, for a few minutes, subjected to another similar electrolysis, but using dilute (1:10) sulfuric acid instead of the chloroplatinic acid. This reduces to metallic platinum any traces of the platinum compound that may have remained within the black platinum coating. After rinsing with water, the electrode is kept under water so that it never dries out.

When a fresh electrode is platinized for the first time, the coating may proceed slowly and turn out more gray than black. If so, the coating may be "removed" and the electrode coated again. The second coating is usually satisfactory. (a) The electrode is wiped off, dried thoroughly in air, and heated in the tip of a small Bunsen burner so that only the tip of the platinum, not its sealed-in end, is directly exposed to the flame. The surface rapidly turns gray and in this condition lends itself easily to another electrolytic coating. The process requires great caution because a crack, even of invisible size, between the sealed-in platinum and the glass would render the electrode useless. It is quite justifiable to warn against this method, as some authors did, although it is very satisfactory if it succeeds. (b) A less objectionable way of removing the first coating is to subject the electrode, as an anode, to electrolysis in strong hydrochloric acid. A well-platinized electrode can be used over a very long time. It should never be touched with the fingers and should always be kept moist. If there is suspicion of contamination with grease it may be successfully cleaned with 10% sodium hydroxide. It may be added that an electrode, even though not really black but gray, serves its purpose satisfactorily when the smallest amount of colloidal palladium is added to the solution to be measured.

B. USE OF COLLOIDAL PALLADIUM

The use of the hydrogen electrode presupposes that the solution to be investigated contains no molecular species which is reduced with any noticeable speed by platinum black loaded with hydrogen. One such substance is always present in ordinary solutions, namely, oxygen. This is removed by the hydrogen, not only mechanically but also by reduction at the black platinum surface. If any constituent of the solution can be reduced by the catalytic action of platinum-hydrogen *e. g.*, an organic dye, no true equilibrium is established before the reduction is complete. This reduction can be greatly accelerated by adding one or a few drops of colloidal palladium solution. Solid colloidal palladium is available commercially in various concentrations of the metal. It contains some protective colloid of protein nature in a concentration low enough not to interfere with the measurement in moderately well-buffered solution. The solid palladium should be dissolved in water so as to contain 1-2% palladium, and about one drop of this diluted solution may be added for each 10 ml. of the solution to be measured. On using a few more drops even a blank platinum electrode fulfills the task of a hydrogen electrode, as has

been shown by Biilmann,⁷ provided one waits for the establishment of a constant potential. This may require more time than with a platinized electrode, and the procedure should not be recommended as a routine method. It may happen that after long use a platinized black electrode is sluggish in establishing a definite potential. In such a case, the addition of palladium is also very helpful.

C. POTASSIUM CHLORIDE BRIDGE

A convenient potassium chloride bridge is made as follows. A doubly bent narrow glass tube fitting tightly into the hole of the rubber stopper, mentioned above, is closed with a ground-glass stopper at the end which is to be immersed in the liquid. The other end of the tube is left open. The protruding part of the stopper should be not wider, but rather somewhat smaller, than the stopper itself (Fig. 6), because it has to be pushed

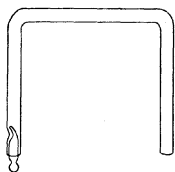


Fig. 6.—KCl-agar bridge.

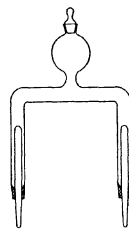


Fig. 7.—Potassium chloride bridge.

through a hole in the rubber stopper. The tube is filled with hot KCl-saturated agar, as described on page 1721. The glass stopper is then tightly inserted so that just the thinnest film of agar is left between the stopper and the wall of the tube. After solidification, this agar bridge is immersed, each end in a separate beaker of saturated aqueous potassium chloride solution covered with a layer of white mineral oil. It is removed from this position only when used. For use, the ends are very quickly sprayed with distilled water and immediately wiped off, and then the tube is inserted as shown in figure 4. Immediately after use the tube is quickly washed with water and immersed again into the saturated potassium chloride solution for recovery. It is best to prepare a set of such tubes and use them successively. From time to time the end with the stopper can be melted and the agar in this end of the tube renewed. One should, from time to time, prepare fresh tubes. A tube that has been in contact with a

⁷ E. Biilmann and A. Klit, *Z. physik. Chem.*, 130, 566 (1927).

solution containing polyvalent ions in an appreciable concentration, or with strong acids or alkalis in high concentration, should be discarded and not put back into the potassium chloride reservoir.

Another type of salt bridge (Fig. 7) is that designed by Irving and Smith,⁸ in which tapered glass rods fit through ground-glass connections. Sintered glass and micro filter tubes have also been successfully used to minimize diffusion from a saturated potassium chloride solution to the solution in the electrode vessel.^{9, 10}

The liquid junction potential at the contact of the potassium chloride bridge with the solution inside the electrode vessel is in most cases not quite constant in time. One may distinguish the state of fresh contact and the final state which is usually reached within half an hour. For instance, using standard acetate and the KCl–agar bridge, the difference in potential with time amounts to about 2 mv. If it should be 517 mv. on fresh contact, it may be 515 mv. finally. In order to study this phenomenon, one may first wait for the establishment of the final potential, and then exchange the agar bridge for a fresh one, while the hydrogen gas is bubbling, and observe the drift of potential with time. The reference value required is, of course, the one used for the same condition when the standard acetate is exchanged for the unknown solution to be measured. In some modifications of the method (see below), the reading with a fresh contact of large area is wisely chosen as the desirable and best reproducible condition. However, for the modification just described, it is safer to measure all potentials after establishing the final value. This is the only reasonable way when not only a single measurement is to be made, but a series, as during an acidimetric titration. Thus, changes in the liquid junction during the titration become negligible. It may be noted that the error of 2 mv. that may be caused by not paying attention to the state of the liquid junction amounts to an error in pH of approximately 0.04. It should, however, be kept in mind that with solutions of very low or very high pH the liquid junction potential, and probably also the drift of it, may be much larger.

D. OTHER TYPES OF HYDROGEN HALF-CELLS

The electrode vessel just described is easy to improvise and is entirely practicable. The same principle has been applied to other devices and modifications, many of which have been published; of these only two examples are shown. Figure 8 represents Hildebrand's¹¹ hydrogen electrode.

⁸ G. W. Irving and N. R. Smith, *Ind. Eng. Chem., Anal. Ed.*, **6**, 480 (1934).

⁹ H. A. Laitinen, *Ind. Eng. Chem., Anal. Ed.*, **13**, 393 (1941).

¹⁰ H. Goto, *Science Repts. Tohoku Imp. Univ., First Ser.*, **29**, No. 1, 9 (1940).

¹¹ H. J. Hildebrand, *J. Am. Chem. Soc.*, **35**, 847 (1913).

It is immersed in the solution to be measured and bubbled with hydrogen gas to the attainment of a constant potential. An improved device is Bunker's¹² design, shown in figure 9, which provides better protection of the electrode from oxygen. Furthermore, Clark's hydrogen electrode¹³ is especially worth mentioning. The whole instrument, including a shaking device and a mechanism for the establishment and convenient renewal of a fresh broad liquid junction between the unknown solution and the saturated potassium chloride solution, is described in complete detail¹³ and is commercially available. The instrument is very elaborately designed and

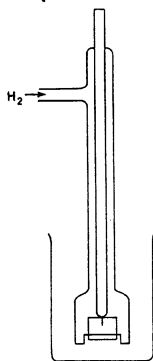


Fig. 8.—Hildebrand's hydrogen electrode.¹¹

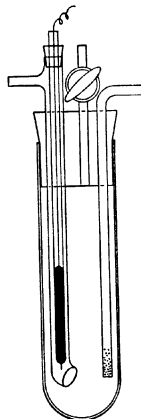


Fig. 9.—Bunker's hydrogen electrode.¹²

particularly useful if one wishes to read the potential with a freshly established liquid junction. The liquid junction can readily be renewed so as to represent the state of a "fresh" liquid junction. The large area and the symmetric shape of the liquid junction provide the best reproducibility of liquid junction potentials. This instrument has been successfully used in many laboratories and is most suitable if one wishes to measure just the pH of a given solution. However, it cannot be used for potentiometric titration where bridges with capillary connections, such as KCl–agar bridges, must be used for which the liquid junction potentials are somewhat less reproducible. However, taking into consideration the comments on

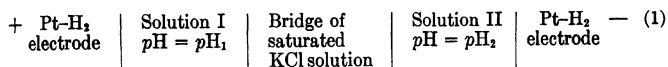
¹² J. W. M. Bunker, *J. Biol. Chem.*, **41**, 11 (1920).

¹³ W. M. Clark, *The Determination of Hydrogen Ions*. 3rd ed., Williams & Wilkins, Baltimore, 1928.

page 1725, such bridges, even though theoretically inferior, will never cause any practical difficulties.

2. Measurements and Calculations. Practical Definition of pH

The definition of pH is best presented in a practical way by a description of the experimental procedure, and the calculation based upon it, by which the pH value is obtained. Let a galvanic cell be presented by the following arrangement:



With the signs of the poles of the cell as indicated, pH₁ is lower than pH₂, i. e., the hydrogen-ion concentration on the left-hand side is the greater. The electrodes consist of platinum, coated with platinum black, and saturated with hydrogen at one atmosphere pressure. The temperature, *T*, in the absolute (Kelvin) scale is the same throughout the whole cell. Then the difference, pH₁ - pH₂, is defined as:

$$pH_1 - pH_2 = \frac{E}{RT/F} \times 2.303$$

where *E* is the electromotive force of the cell, *R* is the gas constant, 2.303 is the modulus converting natural logarithms into common logarithms, and *F* is the electric charge inherent in one mole of a univalent ion, the "faraday" unit, 96,540 coulombs. With numerical values of these constants inserted and *E* measured in volts:

$$pH_1 - pH_2 = E/0.000198 T$$

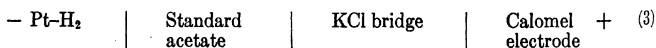
The difference of two pH values thus being defined, there remains to be defined the absolute value of some one pH. This is accomplished in one of the following ways, which are compatible with each other within the limits of experimental error: (a) In an aqueous solution of hydrochloric acid of very low concentration (10⁻³ to 10⁻⁴ *M*) pH equals the common logarithm of the concentration, with reversed sign. So a 10⁻⁴ *N* solution of hydrochloric acid is defined as having pH = 4. A solution of 0.01 *N* hydrochloric acid + 0.09 *N* potassium chloride is supposed to have pH = 2.076 (Sørensen). (b) The pH of standard acetate buffer, i. e., an aqueous solution which is 0.1 molar with respect to acetic acid and also with respect to sodium acetate, is, at 12° C., 4.650; at 25°, 4.640; and at 38°, 4.635; i. e., practically independent of the temperature. The values are those

obtained by MacInnes,¹⁴ and appear to the writer as especially well founded.

Another good reference is a 0.05 *M* solution of potassium acid phthalate, introduced by M. W. Clark. The *pH* of this solution is, according to MacInnes, at 12° C., 4.000; at 25°, 4.000; and at 38°, 4.015.¹⁵ It is very convenient for glass electrodes, but supposedly not perfectly reliable for hydrogen electrodes since phthalate is reduced, though slowly. From this it follows that the *pH* of an unknown solution can be secured by building the galvanic cell (1) with the unknown solution at one electrode and the known solution, *e. g.*, standard acetate, or any of the other standard solutions, at the other. In practice, instead of measuring the potential difference of two hydrogen electrodes, it is more convenient to measure first the potential difference of a hydrogen electrode made with one of the two solutions against a calomel electrode, and then to measure the potential difference of a hydrogen electrode made up with the other solution against the same calomel electrode. The difference between these two *e. m. f.* values is the same as the *e. m. f.* of the above galvanic cell. The actual procedure of measuring *pH*, then, is to measure the *e. m. f.* of cell (2), in which the calomel electrode is always the positive pole:



The potential of the calomel electrode has been compared with that of the standard acetate-hydrogen electrode, and proved to be unchanged in time, by means of cell (3):



An example will show how to arrive at the *pH* value in a given case. Suppose, (1) the potential difference of the calomel electrode against standard acetate has been found to be 0.5170 v., and (2) the potential difference of the calomel electrode and the solution to be measured has been found to be 0.6170 v. Then the difference between the potential of the unknown solution and the potential of standard acetate is 0.1000 v. Therefore, the difference between the *pH* of the unknown solution, *pH*_{unknown}, and that of standard acetate, *pH*_{st. ac.}, is for a measurement carried out at 20° C. (293° K.):

¹⁴ D. A. MacInnes, *Principles of Electrochemistry*. Reinhold, New York, 1939, p. 278.

¹⁵ A careful critical study of this value has been published by W. J. Hamer, G. D. Pinching, and S. F. Acree, *J. Research Natl. Bur. Standards*, 36, 49-62 (1946).

$$\begin{aligned} pH_{\text{unknown}} - pH_{\text{st. ac.}} &= 0.1000/0.000198 \times 293 \\ &= 0.1000/0.0581 = 1.721 \end{aligned}$$

and consequently:

$$pH_{\text{unknown}} = 4.64 + 1.72 = 6.36$$

The result is given in two decimals. It is impracticable to write out the third decimal, even though it may be reproducible experimentally, because of the uncertainty with respect to the liquid junction potential. Since the potential difference of the calomel electrode against the unknown solution is greater than against standard acetate, 1.72 must be taken with a positive sign. If the opposite is true, the negative sign must be taken.

An alternative method of arriving at the pH value is widely used but has no advantage over that explained above. Considering the potential of the normal hydrogen electrode as zero, one may obtain the potential of the calomel electrode as follows. Let ϵ be the e. m. f. of the cell: calomel electrode, hydrogen electrode, both in standard acetate of pH 4.64. Then the potential of the calomel electrode, $E_{\text{cal.}}$, namely, its potential against a solution of $pH = 0$, provided it were possible to build such a cell with negligibly small liquid junction potential, is (in volts):

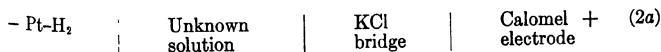
$$E_{\text{cal.}} = \epsilon - 0.000198 \times 4.64 \times T$$

For instance, for 20°C. :

$$E_{\text{cal.}} = \epsilon - 0.0581 \times 4.64 \text{ v.} = \epsilon - 0.2696 \text{ v.}$$

Suppose $\epsilon = 0.5170 \text{ v.}$, as before, then: $E_{\text{cal.}} = 0.2474 \text{ v.}$ In this way, the potential of the particular calomel electrode, at the particular temperature at 20°C. , is established once and for all, provided the electrode remains constant in time.

On measuring cell (2) as above, namely:



at the same temperature and finding its electromotive force = E , it follows that pH of the unknown solution is $pH = \frac{E - E_{\text{cal.}}}{0.000198 T}$, i. e., at 20°C. ,

$$pH = \frac{E - E_{\text{cal.}}}{0.0581}.$$

A list of the values of $0.000198 \times T$ for various temperatures is given in table I.

TABLE I
 VALUES OF $0.000198 \times T$ FOR VARIOUS TEMPERATURES

Temp., ° C.	$2.303 \times RT/F$	Temp., ° C.	$2.303 \times RT/F$
0	0.0542	25	0.0591
4	0.0548	26	0.0593
10	0.0561	27	0.0595
12	0.0565	28	0.0597
14	0.0569	29	0.0599
16	0.0573	30	0.0601
18	0.0577	32	0.0605
19	0.0579	34	0.0609
20	0.0581	36	0.0613
21	0.0583	38	0.0617
22	0.0585	40	0.0621
23	0.0587	45	0.0631
24	0.0589	50	0.0648

A. STANDARDS

Standard acetate is a very convenient standard solution because it is very easy to prepare, it keeps indefinitely without formation of mold or bacterial growth, and its potential in a hydrogen electrode, against the saturated calomel electrode, is practically independent of the temperature within reasonable limits, provided both half-cells are at the same temperature.

It is prepared as follows. An approximately 1 *M* solution of acetic acid is made up and its titer determined accurately by titration with a solution of NaOH of known titer in the neighborhood of 1 *M*, using methyl red as indicator. It is not necessary that the NaOH be strictly free from carbonate. Then 100 ml. of 1 *M* NaOH (or the equivalent amount of the standardized NaOH solution) is mixed with 200 ml. of the 1 *M* acetic acid (or the equivalent amount according to the titer) and made up to one liter with distilled water, which need not be strictly free of carbon dioxide.

B. INFLUENCE OF BAROMETRIC PRESSURE AND TEMPERATURE

In the definition of the idealized normal hydrogen electrode of $pH = 0$, hydrogen is assumed to be present at 1 atm. pressure, that is, the hydrogen gas saturated with water vapor should have a partial pressure of 1 atm. with respect to hydrogen. In the method described above, the barometric pressure will play a role if it is different at the calibration of the calomel electrode against the hydrogen electrode and at the pH measurement proper. A variation of the hydrogen pressure, from p_1 to p_2 will cause a difference in potential:

$$E = \frac{RT}{2F} \times \ln \frac{p_1}{p_2} = \frac{0.00198T}{2} \log_{10} \frac{p_1}{p_2}$$

When the pressure is smaller in the measurement proper than in the calibration, the error makes the potential more positive, and vice versa. The

effect, although small, may often be worth considering and the above correction for the e. m. f. may be applied.

Let us assume that the calibration of the calomel electrode against the standard acetate hydrogen electrode was made at 25° C. and 760 mm. barometric pressure, and the measurement of the unknown solution against the same calomel electrode at the same temperature was made at 750 mm. pressure. At 25°, the vapor pressure of water is 24 mm. Hence, the hydrogen pressure was 736 and 726 mm., respectively, and:

$$\Delta E = \frac{0.000198 \times (273 + 25)}{2} \log \frac{736}{726} = 0.0015 \text{ v.}$$

Since the unknown solution was measured at the lower pressure, this correction must be subtracted from the measured e. m. f. The correction for pH , which must be added to the pH , is:

$$0.0015/0.058 = 0.025$$

If the calibration measurement and the measurement of the unknown solution have been made at slightly different temperatures, in general, a correction will also be necessary. However, if a saturated calomel electrode has been calibrated against standard acetate, one may rely on the agreeable property of this particular calibration system, that its e. m. f., over a reasonable temperature range, is independent of the temperature within the limits of error.

3. Theoretical Foundation of Definition of pH

At the time when galvanic cells of the type described above were first used for the purpose of establishing an acidity scale, it was expected that such cells would permit the measurement of the concentration of hydrogen ion, H^+ , according to Nernst's theory that the potential of a metal electrode depends logarithmically on the concentration of the dissolved ion of the metal. The symbol, pH , was introduced by Sørensen as an abbreviation for $\log 1/[H^+]$. The first refinement of the theory was to replace concentrations by activities. However, there exists an essential and insuperable difficulty in that no experimental operation has been found by which the thermodynamic activity of a single ionic species can be determined with perfect accuracy. What in general can be determined rigidly is only the mean activity of the ions of an electrolyte, which is interpreted as the square root of the product of the activities of the anion and cation. Only if it were possible to measure with strict accuracy the potential of the hydrogen electrode against a solution containing hydrogen ions would the activity of the hydrogen ion be measurable with real accuracy. But this is not possible because any cell containing a hydrogen electrode and some reference

electrode of necessity contains a liquid junction establishing a potential which enters into the electromotive force of the cell. Saturated potassium chloride solution is used as a bridge between the two half-cells because this salt is supposed to decrease as much as possible the liquid junction potential. If the solution in contact with it is of low ionic strength, the potassium chloride bridge may approximately fulfill the purpose but never entirely. If, in working with buffer solutions of low ionic strength in the pH range from 1 to 13, or in the best case, from 0 to 14, the liquid junction were not only small but, what is more important, practically independent of the chemical nature of the solution to be measured, the liquid junction potential would not matter. It would, as it were, always change the potential of the reference electrode to the same extent, and so make it irrelevant. However, this is not so. This flaw in the accuracy should not discourage a critical use of pH values. In most cases the property which depends on the acidity of the solution cannot be determined with any greater accuracy than that corresponding to the uncertainty of the pH value. In any case, to arrive at a practical definition, the concept of pH was defined, at the beginning of this chapter, not in terms of its original thermodynamic significance, but in terms of operations and calculations based on them, which are, of course, closely related to the theory. Such pH values can, for many purposes, be used to represent with reasonable accuracy what may be called the true activity of the hydrogen ion, on a logarithmic scale.

4. Measurement of Concentration (Instead of Activity) of Hydrogen Ions

Under certain conditions the concentration, instead of the activity, of the hydrogen ion can be determined, although not with mathematical accuracy, yet with great precision, from a practical point of view, within the limits of experimental error of potentiometric measurements.¹⁶ The conditions are as follows. Both the known reference solution and the solution to be investigated must contain some suitable electrolyte, such as sodium chloride, at the same concentration and in large excess over the concentration of the buffer which determines the hydrogen-ion concentration of either solution; for example, most solutions encountered in physiologically important experiments are about 0.15 M with respect to $NaCl$ and contain, in addition, a buffer (usually carbonate, phosphate, or protein) at a very much lower concentration. Under such circumstances the activity factor of the hydrogen ion depends, practically speaking, only on the concentration of $NaCl$. Hence, the ratio of the activities of H^+ in two solutions equals the ratio of the concentrations of H^+ . As reference electrode,

¹⁶ D. I. Hitchcock and R. Peters, *J. Am. Chem. Soc.*, **68**, 1753 (1946).

a solution of HCl, say of 0.01 or 0.001 *M* concentration, in a 0.15 *M* NaCl solution, is chosen. HCl being completely dissociated, the concentration of the hydrogen ions $[H^+]$ equals that of HCl. The potential difference of the unknown solution and the reference solution depends, as always, logarithmically on the ratio of the activities of the hydrogen ions. Provided the solution to be measured is also 0.15 *M* with regard to NaCl, the activity factor of H^+ is the same, and the potential difference depends on the *concentrations* of the hydrogen ions. Thus, the method leads directly to the determination of the *true* concentration of the hydrogen ions $[H^+]$ or rather $[OH_3^+]$.

5. Limitations of the Hydrogen Electrode

The hydrogen electrode is not always satisfactory. First of all, there are certain compounds which interfere with the use of platinized platinum, *e. g.*, many of the sulfur compounds, including sulfides and disulfides. Probably complex platinum compounds are formed at the metal surface which disturb the chemical mechanism needed for a hydrogen electrode. In such cases, no steady potentials are obtained and two electrodes in the same vessel may disagree, often considerably. An electrode once spoiled in this way is often regenerated by washing with dichromate-sulfuric acid (cleaning solution). Sometimes it may need resurfacing, and sometimes it may be necessary to discard it altogether. The test for the proper behavior of an electrode is to measure its potential in standard acetate buffer, which easily reveals any improper state of the electrode.

Another occasion where a platinum electrode may become ineffective occurs when solutions containing proteins are measured. A film of denatured protein may gradually be formed. It is easy to regenerate such an electrode by washing with 5% sodium hydroxide. On bubbling hydrogen through protein solutions, addition of a *minute trace* of octyl alcohol counteracts the formation of foam without disturbing the *pH* measurements.

A third cause of trouble occurs when the solution to be measured contains a substance which can be reduced by platinum-hydrogen. For instance, if a solution contains some methylene blue, equilibrium will not be established until the dye is completely reduced. Because of the small catalytically active surface, it may take a very long time to reduce the dye simply by bubbling with hydrogen. If the concentration of the dye is very low, the disturbing influence may be too small to be noticeable; otherwise the reduction can be speeded up satisfactorily by adding a small drop of colloidal palladium. After all, a similar condition prevails essentially in every case, the substance which can be reduced at the electrode, but is not so at the beginning of the experiment, being the dissolved oxygen.

Bubbling with hydrogen has the double purpose of removing oxygen mechanically and reducing the residue. The mechanical removal is never so complete that the minute trace of oxygen left would not interfere. The main purpose of the black coating is to provide a catalyst for the reduction of the last traces of oxygen and other reducible substances.

Sometimes there is a slight difference in the potential when measured while the bubbling with hydrogen is still going on and when it is stopped. In such a case, provided the bubbling has been continued to the attainment of a steady state, the potential of the electrode at rest is preferable to that during the bubbling. For, if there be any reducible substance left, it will be reduced at least at the very surface of the electrode and remain there in the reduced state if not shaken.

6. Glass Electrode

The theory of the glass electrode,¹⁷ in a simplified form which later will be expanded, may be presented as follows. A thin membrane of a soft glass of proper composition acts as a membrane permeable only to hydrogen ions. When such a glass membrane is interposed between two solutions of different pH value, the potential difference across the glass membrane is:

$$E = \frac{RT}{F}(pH_1 - pH_2)$$

In order to measure this potential difference, each of the two solutions is brought into contact with a metal electrode in such a way that either the two metal electrodes are at the same potential or have a constant and known potential difference. All liquid junction potentials should be negligibly small (the potential across the glass membrane is not counted as a liquid junction potential). Suppose the e. m. f. of such a cell is measured, one solution with pH_1 being a known buffer solution, say standard acetate of pH 4.64, and the other any solution of unknown acidity, then the unknown pH , or pH_2 , may be calculated by use of the above equation.

To accommodate this simplified theory to real conditions, the following amendments must be introduced. According to the simplified theory, two equal solutions, with the glass membrane interposed, should show no potential difference. This is not always true. Such a potential, called the asymmetry potential, is usually large in a freshly blown glass membrane and subsides very gradually within days or weeks. However, even for glass membranes showing asymmetry potential, when the pH of one of the two solutions is varied, the other being kept constant, usually, with a mem-

¹⁷ M. Dole, *The Glass Electrode*. Wiley, New York, 1941 (contains a complete bibliography and many valuable discussions with regard to theory and practice of potentiometric methods in general).

brane of the proper composition of glass, the variation of potential with pH is the same as in a hydrogen electrode. Therefore, the following procedure for measuring pH has been devised. One of the two solutions is kept constant all the time. The best way is by use of 0.1 *N* hydrochloric acid in conjunction with a silver-silver chloride electrode (described on page 1737). A potentiometric measurement is made with a known buffer solution, *e. g.*, standard acetate of pH 4.64, on the other side of the glass membrane, by connecting it through a saturated potassium chloride bridge with a saturated calomel electrode. A silver electrode coated with silver chloride cannot be used here because silver chloride is soluble in potassium chloride solution. The electromotive force of this cell is E_1 . When the standard acetate is replaced by the unknown solution, the e. m. f. is E_2 , and the pH of the unknown solution is:

$$pH = \pm \frac{E_2 - E_1}{RT/F} \quad \text{or, at } 20^\circ \text{ C., } pH = \pm \frac{E_2 - E_1}{0.0581}$$

The sign is chosen according to whether the e. m. f. becomes larger or smaller on replacing the known buffer by the unknown solution, "smaller" including those cases in which the direction of the current is reversed, as may happen for very acid solutions. If the e. m. f. becomes smaller, the pH of the unknown solution is smaller than that of the buffer, and vice versa.

In order to check the proper behavior of an individual electrode, namely, to see whether the potential varies linearly with pH , at a slope of 0.0601 v. per pH at 30° C. (or 0.0591 v. at 25° C.), the pH difference of various buffers of known pH should be measured, *e. g.*, a phosphate buffer of pH 6.81, and an acetate buffer of pH 4.64. The difference in pH of 2.17 should correspond to a change in e. m. f. of $2.17 \times 0.591 = 0.1282$ v. (at 25°). A limit of error of ± 0.0005 v. may be allowed. It is best to check from time to time the behavior of the glass electrode in this respect. After long use, the result may be unsatisfactory, and the potentials may even be erratic. A. Beckman suggests a regeneration of the glass surface by exposing it for 3 minutes to the corroding effect of a 30% solution of ammonium difluoride. The glass membrane should never be allowed to dry out. One side is kept permanently in contact with the 0.1 *N* hydrochloric acid, and the other, when not used, is kept in water. A constant potential should be established, although not always quite immediately, certainly within a few minutes.

The glass electrode gives reliable pH values only within a pH range of approximately 1 to 9, reasonably reliable values (too low by a few millivolts) from pH 9 to 10, and approximate but not very reliable values from pH 10 to 11. Above pH 11, the results are decidedly erroneous. The

reason for the limitation of the measurable range is that in solutions containing very few hydrogen ions and a large number of alkali-metal ions, it is no longer true that the membrane is permeable only for hydrogen ions. As shown by MacInnes, the error caused by Na^+ is different from that caused by K^+ , or other metal ions.

Recently, a kind of glass has become available which gives reliable values even in alkaline solutions to the same extent as the hydrogen electrode.¹⁸ The potentials usually require somewhat more time to reach constant values with this type of electrode than with the hydrogen electrode.

Among the shapes of the glass electrode, three types may be mentioned. Of these, two (*b* and *c*, Fig. 10) were introduced by MacInnes. The third type, used by F. Haber, discoverer of the glass electrode (*a*, Fig. 10), is a glass tube, thick-walled in general, and blown out to a very thin-walled bulb. It is filled permanently with 0.1 *N* HCl in which an Ag-AgCl electrode is immersed. Electrode *b* is similar, but instead of having a blown-out lower portion, a small piece of glass cut from a very large, blown-out, very thin-walled glass bulb is sealed across one opening of the tube by heating

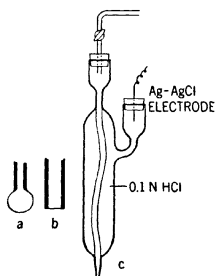


Fig. 10—Three types of glass electrodes.

the tube and, while it is hot, pressing it upon the glass membrane. Both in model *a* and in model *b*, the thick-walled part of the glass is coated with varnish to prevent a short-circuit from the inside to the outside through a moisture film on the glass wall. The very useful type of glass electrode represented by *c* consists of a thick-walled glass vessel with a side arm, and a thin glass tube which is sealed into the vessel. The ends of the tube which protrude from the vessel are of thick-walled glass, and the inside section of thin-walled glass. Only a skillful glass blower can fuse the thick-walled glass portions to the glass membrane tube, by using several transitional glass mixtures for the joint. The large outer tube is permanently filled with 0.1 *N* hydrogen chloride and a silver-silver chloride electrode is placed in the side arm. The inner tube is filled by suction with water or with the solution to be measured. The outlet end of the tube may be equipped with a five-way stopcock, described by MacInnes, which makes it possible to establish fresh contact with a saturated potassium chloride solution or a saturated calomel electrode, and so to establish a fresh liquid junction before the final reading of the potential. As regards using fresh or aged liquid junctions, the same holds, of course, as for hydrogen electrodes.

¹⁸ Beckman E-type electrode of the National Technical Laboratory, South Pasadena, Cal.

In using glass electrodes, much more sensitive instruments are required for the measurement of the e. m. f. than with hydrogen electrodes, because of the high resistance of the glass electrodes, which even with very thin-walled membranes may range from 5 to 100 megohms or even more. Only with electrode model *c* (Fig. 10) may measurements be made with the electrical arrangement described above for use with the hydrogen electrode, provided the dimensions of the glass electrode are large and the glass membrane is thin, so as to make the resistance not much more than 5 megohms. However, even with this electrode, the galvanometer must be very much more sensitive than that used for the hydrogen electrode, say, of a sensitivity of 10^{-11} amp. per mm. of deflection at one-meter distance. This method has the advantage that the instruments need no shielding. The writer has used it for a long time very satisfactorily. On the other hand, the insulation of the leads must be very good; even so, on moist warm summer days stray currents will counteract its usefulness.

The glass electrode, although somewhat restricted with respect to the pH range, is an indispensable instrument for cases in which the hydrogen electrode fails to work, especially if the system contains an easily reducible substance such as a ferric compound, or dichromate, or a reducible dye-stuff, even in high concentration. While the hydrogen potential is established only after the reduction of such substances—which is often not easily accomplished without changing the original pH—the glass electrode is unaffected by their presence. Much of the commercially available equipment requires very little material, say 1 or 2 ml., and some even less, of the solution. The glass electrode can be used for acidimetric titration within a pH range of from 1 to 8 or 9, with accuracy, and with approximate accuracy to 10. So, it can be successfully used for titrations of not too weak acids, with $pK \lesseqgtr 6$. A solution is titrated with the standardized alkali (or acid) and after each addition a pH measurement is carried out. If an electrode of type *c* is used after each addition of standardized alkali, a portion is sucked into the electrode, and after each pH measurement is poured back into the bulk of the solution contained in a beaker. A KCl-agar bridge is permanently immersed in the beaker for connection with the calomel electrode. In some cases it may be necessary to shield electrically the whole apparatus, including the burette system, by covering it with a hood of metal screening which is grounded.

7. Silver-Silver Chloride Electrode

For the method described here, based on the calibration of the electrode with standard acetate, it is not necessary to prepare the silver electrodes so as to give the best reproducible values. It is sufficient to have some silver electrode with a constant potential. This is easily obtained as fol-

lows. A platinum electrode as described on page 1721 (a wire will do; a plate is not necessary) is cleaned with hot nitric acid, washed, and coated with silver by cathodic polarization in a solution of silver cyanide, with a few milliamperes, over many hours, to secure a thick silvercoating. The silver cyanide should be freed from excess potassium cyanide by recrystallization. Then the electrode, preferably after brief rinsing in concentrated ammonia to remove excess cyanide, is washed in running distilled water. This washing should be carried out for some days, unless the electrode has been rinsed in ammonia, which cuts the washing time to a few hours. Thereafter, the electrode is electrolyzed, as anode, in a dilute hydrochloric acid solution with one or a few milliamperes, and is washed thoroughly for a long time in distilled water.

The 0.1 *N* hydrochloric acid in the glass electrode as described is used with more convenience in conjunction with a silver-silver chloride electrode than with a calomel electrode, because the silver-silver chloride electrode is just a wire coated with silver which can be simply submerged and permanently kept in the hydrochloric acid solution. However, the silver electrode cannot always be used as a substitute for the calomel electrode, because silver chloride is soluble in saturated potassium chloride solution. A calomel electrode is therefore used as the other half-cell.

8. Quinhydrone Electrode

The quinhydrone electrode was first used for *pH* measurements by Büllmann.^{19,20} It is based on the dependence of an oxidation-reduction potential on *pH*. It is of considerable interest, although its practical use is being superseded more and more by the glass electrode. A mixture of quinone and hydroquinone in contact with a bright platinum (or gold) electrode establishes a potential which depends linearly on *pH* within the *pH* range from strongly acid solution, say *pH* 0, to a *pH* of about 7 or 7.5. At *pH* > 7.5, no steady potentials are obtained for quinhydrone for reasons which will be given below.

To the solution to be measured quinhydrone is added in an amount not too small, but otherwise arbitrary. Quinhydrone is a well-crystallized molecular compound of quinone and hydroquinone, which, in solution, dissociates almost completely into quinone and hydroquinone. The original yellowish brown color of the solution gradually turns darker, slowly in acid, but rapidly in alkaline solution, due to irreversible changes of the quinone. In acid solution, this decomposition is slow enough not to interfere essentially with the use of the quinhydrone electrode. In alkaline solution, not only is the quinone quickly disintegrated, but in the presence of

¹⁹ E. Büllmann and M. H. Lund, *Ann. chim.*, **15**, 111, 321 (1921).

²⁰ E. Büllmann, A. Klit, and T. Swaetichin, *Biochem. J.*, **22**, 845 (1928).

oxygen the hydroquinone is oxidized. It is essential that the solution to be measured contains equimolar amounts of quinone and hydroquinone. Theoretically, their absolute concentration should not matter. In practice, however, because of the lability of the substance, it is preferable always to use enough quinhydrone to make nearly a saturated solution. The addition of quinhydrone, within the pH range in which it can be used, does not change the pH of the solution, because quinone for all practical purposes is neutral, and the ionization constant of hydroquinone is so small that it remains practically unionized at $pH < 10$. In general, removal of the dissolved oxygen would be of little importance, and no bubbling with any gas is necessary. It should be noted, however, that driving out of the dissolved oxygen by nitrogen may cause slight differences in the potentials. However, this procedure would annihilate the convenience of the quinhydrone electrode in comparison with the hydrogen electrode, namely, the avoidance of the use of gases.

The simplest procedure for measuring pH with the quinhydrone electrode is as follows. A quinhydrone electrode is made up with standard acetate of pH 4.64 and its potential measured against the saturated calomel electrode, using a saturated potassium chloride bridge for the liquid junction. The potential of the quinhydrone electrode is very much more positive than that of any hydrogen electrode, even in a very acid solution. The potential should be measured as it is, not relying on any tabulated standard value. Then a quinhydrone electrode is made up with the unknown solution and its potential is measured at the same temperature $\pm 0.5^\circ$ against the calomel electrode. The difference, Δ , in volts, between the two e. m. f. measurements is related to the difference between the unknown pH and the pH of the standard acetate (4.64) as follows:

$$pH_{\text{unknown}} - pH_{\text{st. ac.}} = \pm E/0.0581 \text{ (at } 20^\circ \text{ C.)}$$

The plus sign is chosen when the potential of the standard acetate is less positive than that of the unknown, and vice versa. The factor 0.0581 must be changed according to the temperature, as shown in table I, page 1730.

Establishment of the potential is quite rapid. However, there is never, especially in less acid solutions, a potential that might be considered as strictly stable for any length of time, although the change, due to decomposition, is usually slow in acid solution. Good judgment must be used for the proper time of the final readings. The method is convenient for many purposes if used critically, but the claim for its accuracy has been somewhat exaggerated. Appreciable errors may result if the solution to be measured contains substances which are oxidized by quinone or reduced by hydroquinone.

Measurements of pH with the quinhydrone electrode should agree with those obtained with the hydrogen electrode for solutions of very low ionic strength. According to Bilmann, the potential (in volts) of the quinhydrone electrode against the hydrogen-gas electrode at the same pH is between 0° and 38° C.:

$$e = 0.699 - 0.00074 (25^\circ - t^\circ)$$

At higher ionic strengths, the same "salt error" may occur as with pH determinations by means of indicators. A procedure to avoid this error has been established by Sørensen.²¹

Commercial quinhydrone is usually prepared from hydroquinone by partial oxidation with a ferric salt, and may contain some iron. An iron-free quinhydrone is easily prepared by mixing equal amounts of quinone and of hydroquinone. Each is separately dissolved in a small volume of alcohol, the hydroquinone solution is diluted with water, and the quinone solution is added gradually. The black crystalline precipitate is collected at the pump, washed with water, and dried in a desiccator. Finely powdered, it is added to the solution to be measured, and stirred, in order to obtain an approximately saturated solution.

Analogous to the use of quinhydrone is that of tetrachlorohydroquinone and tetrachloroquinone ("chloranil"). The solution to be measured is thoroughly shaken, to saturation, with both substances. Therefore, in the resulting chloranil electrode, introduced by Conant and Fieser,²² the partners of the oxidation-reduction system are present in different concentrations. With the original quinhydrone electrode it is not practicable to saturate the solution both with quinone and with hydroquinone because of the high solubility of hydroquinone. The advantage of the saturated solution is that any "salt error" is eliminated. The chloranil electrode can be used in nonaqueous solvents such as glacial acetic acid. The potential of the chloranil electrode is $+0.664$ at $25^\circ + 0.059 \log [H^+]$ according to Conant.²²

IV. POTENTIOMETRIC ACIDIMETRIC TITRATION

1. Theory and Definitions

Potentiometric titration^{23,24} consists in adding from a burette, step wise, small portions of a standardized strong acid such as hydrochloric acid, or a strong base, such as sodium hydroxide, and measuring the pH at each step. In no case is there any advantage in titrating a solution

²¹ S. P. L. Sørensen and K. Linderstrøm-Lang, *Ann. chim.*, **16**, 283 (1921).

²² J. B. Conant and L. F. Fieser, *J. Am. Chem. Soc.*, **45**, 2194 (1923). J. B. Conant, L. F. Small, and B. S. Taylor, *J. Am. Chem. Soc.*, **47**, 1959 (1925).

²³ I. M. Kolthoff and N. H. Furman, *Potentiometric Titration*. Wiley, New York, 1931.

²⁴ N. H. Furman, *Ind. Eng. Chem., Anal. Ed.*, **14**, 367 (1942).

with a weak instead of with a strong acid or base; on the contrary, all end points of titration become less sharp when titrating with a weak acid or base.

The pH readings are plotted against the amount of standard acid (or base) added. The curve obtained can be interpreted with respect to the end point of titration, which has the same significance as the end point found by color change in titrations with indicators, and with respect to the determination of the ionization constants (dissociation constants) of acids or bases contained in the solution. In the first approximation the shape of the potential curve may be said to depend only on the chemical nature and quantity of the acid or base, and to be independent of any salts contained in the solution. This, however, is true only if the ionic strength of the solution is very low and remains so during the titration. The ionic strength, μ , of a solution is defined as follows:

$$\mu = \frac{1}{2} \sum_{i=1}^{i=n} c_i n_i^2$$

where c_1, c_2, \dots, c_n is the concentration and n_1, n_2, \dots, n_i is the valency of each ionic species. For instance, in a 0.1 *M* solution of sodium chloride, (Na^+ 0.1 *M*, Cl^- 0.1 *M*, valence of either species 1):

$$\mu = \frac{1}{2} (0.1 \times 1^2 + 0.1 \times 1^2) = 0.1$$

The ionic strength equals the molar concentration of sodium chloride. In 0.1 *M* calcium chloride solution:

$$\mu = \frac{1}{2} (0.1 \times 2^2 + 0.2) = 0.3$$

Only when μ is very small, say ≤ 0.01 during the whole titration, is the shape of the titration curve almost independent of the concentration of the solution. The end point, however, is independent of the ionic strength. Therefore, the ionic strength is irrelevant if the titration is carried out just in order to determine the end point.

2. Examples of Titration Curves

The end point can be recognized by the sharp rise in the slope of the titration curve. The following examples will show the way in which the end point manifests itself for acids of various strengths. We use the term "acid" according to the general definition, *i. e.*, for any molecular species which can detach a proton.²⁵ In the old nomenclature, these species

²⁵ Quite justly, objection has been raised to calling a molecular species such as NH_4^+ an acid. The expressions "proton donor" and "proton acceptor" instead of "acid" and "base" may be more appropriate. However, the somewhat unorthodox new definition, proposed by Brønstedt, may be accepted until a universal nomenclature has been agreed upon.

would either be called free acids, such as hydrochloric acid or acetic acid or salts of bases, such as ammonium chloride. The latter is a mixture of the two ionic species, NH_4^+ and Cl^- , of which Cl^- is unaffected during the titration. NH_4^+ , however, can detach a proton, and, when sodium hydroxide is added, the reaction, $\text{NH}_4^+ + \text{OH}^- = \text{NH}_3 + \text{H}_2\text{O}$, takes place. The ionization constants in the old sense, k_a or k_b , for an acid or a base, respectively, and those in the new sense, K_a or K_b , are related to each other as follows:

$$K_a = k_a$$

$$K_b = \frac{k_w}{k_b}$$

where $k_w = [\text{H}^+][\text{OH}^-]$, the ionization product of water. The numerical values for k_w in water or aqueous solution of very low ionic strength for different temperatures are shown in table II, according to the writer's earlier measurements.

TABLE II
IONIZATION CONSTANT OF WATER, k_w

Temp., ° C.	$\log \frac{1}{k_w} = p k_w$	pH of neutral point
16	14.200	7.10
18	14.130	7.07
20	14.065	7.03
22	13.995	7.00
24	13.925	6.96
26	13.860	6.93
28	13.890	6.90
30	13.725	6.86
32	13.660	6.85
34	13.600	6.80
36	13.535	6.77
38	13.475	6.74
40	13.420	6.71

In recent years, both the theoretical background and the experimental procedure for the determination of the ionization constant of water have been greatly refined. Table III gives the data published by Harned and Hamer.²⁶ Their definition of the ionization constant of water, K_w , is:

$$K_w = \frac{\gamma_{\text{H}}\gamma_{\text{OH}}}{a_{\text{H}_2\text{O}}} m_{\text{H}}m_{\text{OH}}$$

where γ_{H} and γ_{OH} are the activity coefficients of the H^+ and OH^- ions, respectively, m_{H} and m_{OH} their concentrations in terms of molality, and

²⁶ H. S. Harned and W. J. Hamer, *J. Amer. Chem. Soc.*, **55**, 2194 (1933).

$a_{\text{H}_2\text{O}}$ the activity of water, which is taken as equal to 1 for the case of ionic strength $\mu = 0$.

Although these figures are very accurate, the practical advantage in the utilization of their accuracy is often hampered by the fact that γ and α are usually not known with sufficient accuracy under most of the experimental conditions.

The unknown solution may either be that of an acid, and titrated with sodium hydroxide, or of a base, and titrated with hydrochloric acid. In the following discussion, we shall consider the titration of a solution of an unknown acid with a standardized solution of sodium hydroxide. By the use of the generalized definition, the theory of the titration of an unknown base is analogous and needs no special comment. It is necessary to take into consideration whether the acid is a strong one, such as hydrochloric acid, which dissociates completely, or a weak one having an ionization constant of from 10^{-3} to 10^{-8} , *e. g.*, acetic acid, or a very weak acid having an ionization constant of 10^{-8} to 10^{-10} , such as boric acid. Acids of

TABLE III
IONIZATION CONSTANT OF WATER, K_w

Temp., ° C.	$K_w \times 10^{14}$	pK_w
0	0.115	14.939
5	0.186	14.730
10	0.293	14.533
15	0.452	14.345
20	0.681	14.167
25	1.008	13.997
30	1.471	13.833
35	2.088	13.680
40	2.916	13.535
45	4.016	13.397
50	5.476	13.262
55	7.297	13.137
60	9.614	13.017

still smaller ionization constants show no reliable end point of titration in aqueous solution.

Titration of Strong Acid.—Hydrochloric acid, say 20 ml. of a 0.01 *M* solution, is titrated with sodium hydroxide. The concentration of the latter should be so chosen that the volume change of the solution is not too great up to the end point of titration, *e. g.*, a 0.1 *M* solution of sodium hydroxide, free of carbon dioxide, may be added from a finely graduated burette. After each addition, the hydrogen-ion concentration equals the concentration of that part of the hydrochloric acid which is not neutralized. The titration curve has the shape shown in figure 11. The slope always increases with the increasing value of the abscissa, but there is a very

steep rise at pH 7. Later on, the slope decreases again. Here, the pH of the excessive sodium hydroxide is being measured. The point of inflection is considered as the end point of titration. The rise at this point is so steep that the error in the estimation of the end point in terms of milliliters of sodium hydroxide is negligibly small, unless the molar concentration of the acid to be titrated is extremely low, $\ll 10^{-3} M$.

Titration of Weak Acid with Ionization Constant $K = 10^{-3}$ to 10^{-8} ($pK = 3$ to 8), *e. g.*, acetic acid ($pK = 4.6$).—As shown in figure 12, the titration curve begins with a rise of the curve (more or less distinct according to the concentration of the acid) which flattens out, and the end point

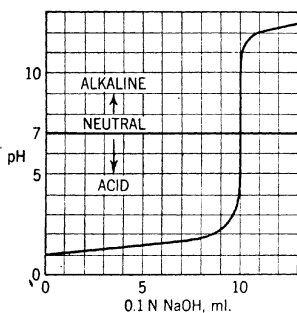


Fig. 11.—Titration of hydrochloric acid with sodium hydroxide.

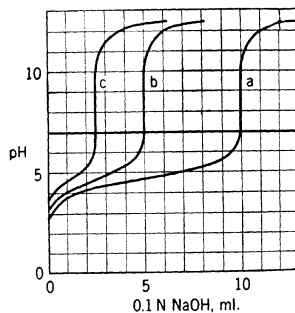


Fig. 12.—Titration of acetic acid with sodium hydroxide, *a*, *b*, and *c* referring to different concentrations of the acid.

is, again, characterized by a sharp rise, not as high as with hydrogen chloride, but sufficiently steep for the determination of the end point. The pH at the 50% point of titration equals the pK of acetic acid. The flat part of the curve manifests the buffer effect of the acetic acid-sodium acetate mixture. Here, the pH of the solution is relatively resistant with respect to the addition of more sodium hydroxide. At the 50% point of titration, $[CH_3COO^-] = [CH_3COOH]$, and, according to the definition of K :

$$K = \frac{[CH_3COO^-][H^+]}{[CH_3COOH]_{total} - [CH_3COO^-]}$$

At this point, $K = [H^+]$ or $pK = pH$. If the same titration is performed after first adding sodium chloride to the acid solution, to make a concentration of sodium chloride of about $0.1 N$, the end point of titration is the

same, but the 50% point is somewhat displaced, resulting in a slightly different value for pK . This phenomenon shows the influence of the ionic strength on the apparent value of pK . Usually, we mean by pK the value extrapolated to infinitely low ionic strength. On titrating acids with a strong base, or vice versa, the ionic strength increases during the titration. The pK obtained from the 50% point of titration is that for the ionic strength at this point. Only if the ionic strength remains small (≈ 0.01) will the error so caused be practically irrelevant.

Titration of a Very Weak Acid, *e. g.*, boric acid ($pK = 8.5$).—In figure 13, the unbroken curve differs from the preceding example in that the end point of titration is even less distinct. The point of inflection may be as poorly defined as indicated by this curve in figure 13. For very weak acids,

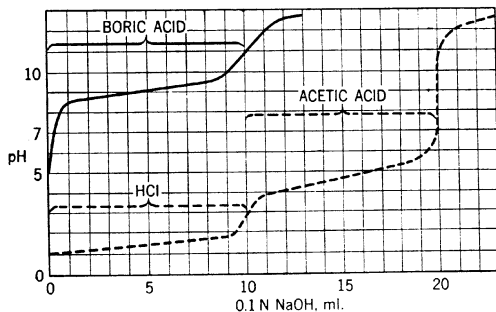


Fig. 13.—Titration of boric acid and of a mixture of hydrochloric and acetic acids with sodium hydroxide.

with $pK > 10$, the end point may be so vague that no accurate interpretation of the titration curve is possible either with respect to the end point or to pK .

Mixtures of Acids, and Polybasic Acids, Including Ampholytes.—When the solution to be titrated is a mixture of a strong acid and a weak one, the titration curve is as shown by the broken line in figure 13. Here, the end points of titration for the two acids are indicated, each by a sharp rise of the potential. However, the rise of the potential at the end point of hydrochloric acid is much smaller than it would be for a pure solution of the acid. The sharpness of the first point of inflection depends on the pK of the weak acid. This can be seen by comparing the titration curve of the mixture of hydrochloric acid and acetic acid ($pK = 4.6$) with that of the mixture of hydrochloric acid and lactic acid ($pK = 3.5$) (Fig. 14). If

the weak acid is even stronger than lactic acid, *e. g.*, tartaric acid, the end point of the hydrochloric acid titration may scarcely be recognizable. Such cases may occur under the following conditions. Suppose it is difficult to prepare some weak organic acid in a pure state, while its sodium salt is prepared more easily. A solution of the sodium salt is made up and an excess of hydrochloric acid is added. This solution is titrated with standard sodium hydroxide. The titration curve manifests the end of the titration of hydrochloric acid, which is also the starting point of the titration of the weak acid; and finally it shows the end point for the weak acid.

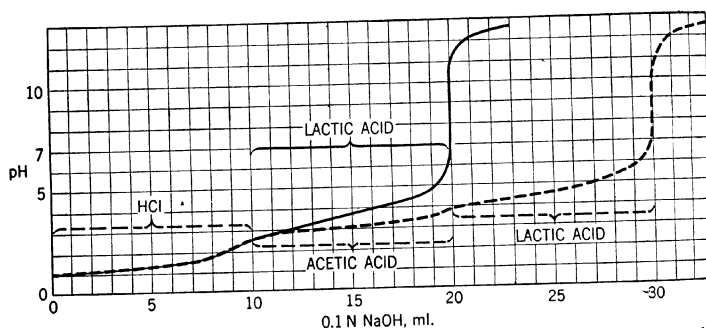


Fig. 14.—Titration of a mixture of hydrochloric and lactic acids (unbroken line) and of a mixture of hydrochloric, acetic, and lactic acids with sodium hydroxide (broken line).

So far, we have considered a mixture of a strong and a weak acid. If two weak acids are present in a mixture to which an excess of hydrochloric acid is added, the titration curve may have the shape shown by the broken line in figure 14 for a mixture of hydrochloric, acetic, and lactic acids. The end of the titration of acetic acid, and so the beginning of the curve for lactic acid, can just be recognized. The distinctness of the separation of the two weak acids depends upon the ratio of their ionization constants. If this is large, *e. g.*, 100 (or $pK_2 - pK_1 = 2$), the jump is quite distinct. If the ratio is ≈ 16 , there is no break at all, and the two curves overlap without any point of inflection occurring. In such a case, it is, theoretically speaking, possible to calculate the two pK values on a principle similar to that to be discussed in the section on oxidation-reduction potentials (pages 1773 *et seq.*). However, this procedure is difficult, and for various reasons not as reliable as in the field of oxidation-reduction potentials.

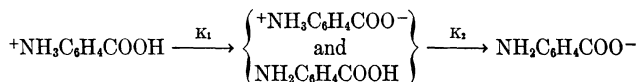
A dibasic acid behaves during the titration in the same way as a mixture of two different acids at equal concentration. The two ionization constants can be easily determined whenever $K_1 > 16 K_2$. A theoretical

statistical consideration shows that, for a dibasic acid, K_1/K_2 cannot be < 4 , and this limiting value is approached only in extremely rare cases. Usually K_1/K_2 is $\gg 16$, and then the determination of the two successive ionization constants causes no difficulty.

An ampholyte can be conceived of as a dibasic acid. For instance, for aminoacetic acid, we may start with its hydrochloride which, in solution, contains the positively charged ion $^+\text{NH}_3\text{CH}_2\text{COOH}$. This is, according to the general definition, an acid, and, for that matter, a dibasic acid which, on adding sodium hydroxide, first detaches one proton and then another. The first proton to be detached is, for any aliphatic amino acid, the one in the carboxyl group. In aromatic amino acids such as *p*-aminobenzoic acid, a mixture of two molecular species is formed on ionization. In part, the proton of the COOH group, in part that of the NH_3^+ group is detached. For glycine, the two steps of ionization are:



For aminobenzoic acid, they are:



In the latter case the constant, K , is the sum of two constants each pertaining to a different kind of ionization, *i. e.*, if we denote with ⁽¹⁾ and ⁽²⁾ the individual ionizations of the NH_3^+ and the CO_2H groups, $pK_1 = p(K_1^{(1)} + K_1^{(2)})$, but not $= pK_1^{(1)} + pK_1^{(2)}$! It is only this sum, $K_1^{(1)} + K_1^{(2)}$, or what we call the apparent or practical ionization constant, k_1 , that can be determined with this method. A separate determination of $K_1^{(1)}$ and $K_1^{(2)}$ may sometimes be possible by spectrophotometric methods if the two isomers have different absorption spectra.

The isoelectric point of an ampholyte is the *pH* at which the concentration of the positively charged ion is equal to that of the negatively charged ion. It is indicated by the point of inflection in the middle of the titration curve. If $K_1 \gg K_2$, as for glycine, the concentration of each of these species is vanishingly small at the isoelectric point where the ampholyte consists practically entirely of the zwitterion.

For an ampholyte with one basic and one acidic group (such as aminoacetic acid), K_2/K_1 can never be smaller than 4; or taking the old nomenclature, k_b/k_a can never be less than $4/k_w$.

Determination of Dissociation Constant of an Acid or a Base.—The dissociation constant of an acid cannot always be determined as simply as mentioned above, *i. e.*, by taking the *pH* at the 50% point of titration as the *pK* of the acid. This method is permissible only for acids of

medium strength such as acetic acid which fulfill the following conditions: (1) in a mixture of the acid with its sodium salt, the number of anions contributed by the dissociation of the acid must be negligible in comparison with the number of anions arising from the dissociation of the sodium salt; and (2) the sodium salt must not be hydrolyzed to any appreciable extent. Very strong or very weak acids do not fulfill these conditions. A more general method for the determination of dissociation constants of acids follows:

(a) If the acid is relatively strong (dissociation constant up to about 10^{-2}), the sodium salt is titrated with HCl and, as a blank, pure water is "titrated" with HCl of the same titer. For both titrations pH is plotted against the amount of HCl added. For any two points corresponding to the same amount of HCl added, there will be two values of pH. On the simplified assumption that $pH = -\log [H^+]$, where brackets mean *concentration*, one obtains two values of $[H^+]$. The difference of the two equals the "amount of HCl neutralized by the sodium salt of the acid," or in other words, the amount of undissociated acid, $[AH]$. Since the total amount of the acid (as used in the form of its sodium salt) is known, $[A^-]$ is also known and all data are available to calculate k from the following equation:

$$k = \frac{[A^-][H^+]}{[AH]} \quad (1)$$

(b) If the acid is relatively weak (dissociation constant down to about 10^{-12}), a solution of the acid is titrated with NaOH of known titer, strictly free from carbon dioxide; as a blank, pure water is titrated with the same NaOH. Proceeding as in the previous case, the difference in $[OH^-]$ for two corresponding points can be calculated using the values of table III (page 1743). This difference equals $[A^-]$, and k can be calculated according to equation (1).

It should be kept in mind that these methods, although useful for obtaining approximate values of k , neglect any difference between concentrations and activities. For greater accuracy corrections for this difference may be worth considering. It should also be kept in mind that the "apparent" k of an acid depends on the ionic strength.

In order to measure the dissociation constant of a weak base a method similar to that used for a strong acid is employed; for a strong base, the method resembles that described for weak acids. Inasmuch as in such titrations a free acid behaves like the hydrochloride of a base, and the sodium salt of an acid behaves like a free base, it is desirable to have a criterion to distinguish an "acid" from a "base" (in the old sense; compare page 1742). Provided the generalized definition of an acid is used, and the dissociation constant is always used in the "new sense," the follow-

ing rule appears to hold generally.^{26a} On comparing pK as obtained from titration in an aqueous solution with that obtained from titration in an alcohol-water mixture, the pK due to an amino group is very little affected by alcohol, whereas the pK due to a hydroxyl or carboxyl group increases considerably with increasing alcohol concentration. In this manner one may also recognize, on titration of an amino acid, which of the two pK values belongs to the amino group and which to the carboxyl group.

3. Technique of Titration

The electrode vessel as described on page 1721, with the addition of a few items, can be used for acidimetric titration. The standardized solution of hydrochloric acid or, as the case may be, sodium hydroxide, is added from a burette, the tip of which is inserted through a hole in the stopper of the titration vessel. If a hydrogen electrode is used, hydrogen is bubbled in the usual way until a steady potential is established, before adding the first portion from the burette. After each addition from the burette, hydrogen is bubbled in again. Once the solution is saturated with hydrogen gas, it takes usually only a short time to reach a steady potential again.

One of the essential precautions to be taken is the avoidance of the presence of carbon dioxide, which acts as an acid at $pH > 4$. When the initial solution to be titrated is strongly acid, the bubbling with hydrogen takes care of the removal of carbon dioxide too. Otherwise, the water used as a solvent should be previously boiled in a Pyrex glass beaker or in a tin-plated copper flask, and, protected by a soda-lime tube, cooled down to room temperature. The sodium hydroxide solution used to fill the burette is freed from carbon dioxide in the following manner. A large flask is filled with a saturated solution of sodium hydroxide pellets (not potassium hydroxide), with a large excess of solid hydroxide. The flask is frequently shaken to reach saturation and is then kept at rest for a period of several days to permit sedimentation of sodium carbonate, which is insoluble in saturated sodium hydroxide. A portion of the clear supernatant solution can be withdrawn with a pipette and properly diluted, while contact with air is avoided as much as possible.

As regards the falsifying effect of carbon dioxide, good evaluation of the reactions involved will indicate the proper procedure. For instance, if the solution to be titrated is alkaline, it will absorb carbon dioxide from the air which cannot be removed by the subsequent bubbling with hydrogen. If the solution is acid, only extremely little carbon dioxide is absorbed, and this is driven out on bubbling with hydrogen.

^{26a} L. Michaelis and M. Mizutani, *Z. physik. Chem.*, **116**, 135-159 (1925). M. Mizutani, *ibid.*, **116**, 350-358 (1925); **118**, 318-326, 327-341 (1925).

DIFFERENTIAL TITRATION

If a potentiometric titration is carried out only in order to obtain the end point of titration and not the whole curve, a method designated as differential titration affords a high degree of accuracy. It was first used by Cox²⁷ and elaborated by MacInnes²⁸ and his associates. The method is based on the fact that the titration curve shows a point of inflection at the end point; in other words, its differential quotient has a maximum at the end point. A titration vessel like the one shown in figure 15 is used; it contains two platinized platinum electrodes, one immersed directly in the solution to be titrated, the other surrounded by a cylindrical tube with a narrow opening. On the addition of a drop of sodium hydroxide, the bulk of the solution will change with respect to pH, but the enclosed

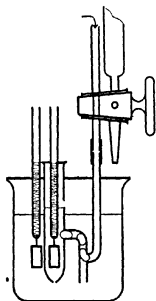


Fig. 15.—Electrode vessel for differential titration, according to MacInnes.

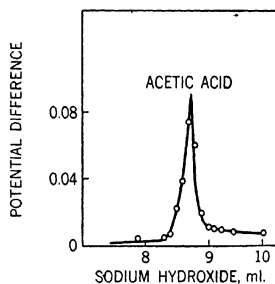


Fig. 16.—Differential titration of acetic acid.

part, which is not immediately mixed with the bulk, will not. So a slight potential difference thus arises. The whole content of the vessel is then thoroughly mixed by bubbling with hydrogen through the narrow tube, in order to obtain a homogeneous solution throughout, and more sodium hydroxide is added. On plotting the potential difference against the differentials of hydroxide added (Fig. 16), there will be a sharp maximum which corresponds to the end point. The advantage of the method lies

²⁷ D. C. Cox, *J. Am. Chem. Soc.*, **47**, 2138 (1925).

²⁸ D. A. MacInnes and P. T. Jones, *J. Am. Chem. Soc.*, **48**, 2831 (1926). D. A. MacInnes, *Z. physik. Chem.*, **A130**, 217 (1927). D. A. MacInnes and M. Dole, *J. Am. Chem. Soc.*, **57**, 1119 (1929). D. A. MacInnes and I. A. Cowperthwaite, *J. Am. Chem. Soc.*, **53**, 555 (1931).

in its high precision, together with the fact that the solution is not contaminated during the titration by diffusion from any salt bridge.

4. Acidity Scale in Nonaqueous Solutions

So far, we have been dealing only with aqueous solutions in which the acids or bases are present in small amounts compared with the solvent, which is supposed to be pure water. When this is no longer true, a number of modifications must be applied to the theoretical basis of pH measurements and even to the definition of pH .

Let us suppose we have a number of solutions all in the same solvent—either water, or a mixture of water with organic solvent, or a water-free organic solvent. This involves tacitly the assumption that all these solutions contain the solvent in such a large excess over the solute that, by adding the solute to the solvent, the character of the solvent is not appreciably changed. However indefinite this statement may be, it coincides with the concept of a dilute solution, which itself is not well defined. One important property which makes a solution a dilute one is that, by adding the solute, the vapor pressure of the solvent is changed by no more than a few per cent. Let us compare dilute solutions of benzoic acid and of salicylic acid, both in 90% alcohol. In this case, there is no objection whatever to adhering to the definition for the *difference in pH* as given for an aqueous solution. One can measure first the potential, E_1 , of one solution against the aqueous calomel electrode, then that of the other, E_2 , and then (at 25° C.):

$$pH_1 - pH_2 = (E_2 - E_1)/0.0591$$

One must, however, take into consideration that the unsolved problem concerning the liquid junction may interfere much more than for an aqueous solution with the thermodynamic significance of this definition.

The contact of the unknown solution with the saturated aqueous potassium chloride solution may be established in two different ways according to whether the organic solvent is miscible with water or not. If it is miscible, a liquid junction potential is established of which we know even less than of any liquid junction potential between purely aqueous solutions. If, on the other hand, the organic solvent is not miscible with water, then a phase boundary is established, and with it a phase boundary potential, about which we know still less. There is good reason to suppose that, on using a bridge of saturated aqueous potassium chloride solution, these incalculable potentials are not exceedingly high, and that they are almost alike on comparing two solutions as similar as in the examples given. However, there is no way of determining any absolute value of pH , for pH

is meant to be the negative logarithm of the activity of the proton. In an aqueous solution, the proton will be present practically entirely, in the hydrated form, as the oxonium ion: $H^+ + H_2O \rightleftharpoons H_3O^+$. Since $[H^+]$ is proportional to $[H_3O^+]$, this fact is irrelevant. All activity factors are undetermined with respect to an arbitrary factor, and the choice of the normal aqueous hydrogen electrode potential as the zero point of potential means to fix that arbitrary factor so that, in a very dilute aqueous solution of hydrochloric acid, $pH = -\log [HCl]$. In an organic solvent, the ratio of $[H^+]$ to $[H_3O^+]$ may be quite different, and complex compounds of the protons, other than $[H_3O^+]$, may exist. Therefore, in organic solvents, only the pH difference can be measured. Of course, pH may be expressed as for aqueous solutions, taking the aqueous normal hydrogen electrode potential as zero. However, it is no longer meaningful to say that, for instance, some solution of pH 1 with glacial acetic acid as solvent is "more acid" than some aqueous solution of pH 2. A reasonable scale of acidity in terms of pH can be established only for solutions in the same solvent.

This insuperable difficulty does not preclude the use of organic solvents for acidimetric titration. Many extremely weak acids which give no satisfactory end point in aqueous solution behave as moderately strong acids in glacial acetic acid and give good end points. Hall and Conant²⁹ succeeded in titrating such a weak base as urea in glacial acetic acid with perchloric acid in the same solvent, which means that urea, under these conditions, can accept a proton. The property that the end point of titration is sharper in some organic solvent than in water was first utilized in the titration of amino acids and especially of peptides or proteins in alcohol³⁰ or acetone,³¹ using suitable indicators. Potentiometric titrations on the same principle may sometimes be useful for the same reasons or because of other features of the titration curve which cannot be observed in aqueous solution.³²

5. Generalized Use of the Term pH for Practical Purposes

Sometimes the concept of pH is used in organic chemistry in a looser sense, not as much concerned with its theoretical significance as to give a practical, reproducible procedure for the preparation of a compound. This is especially the case when dealing with solutions in media other than pure water, *e. g.*, with alcohol-water mixtures. The procedure may read: "Now adjust the pH to 6.8 by addition of such or such buffer." Sometimes, the required accuracy in the pH value is not very high, and then no further

²⁹ N. F. Hall and J. B. Conant, *J. Am. Chem. Soc.*, **49**, 3047 (1927).

³⁰ R. Willstätter and E. Waldschmidt-Leitz, *Ber.*, **54**, 2988 (1921).

³¹ K. Linderström-Lang, *Z. physiol. Chem.*, **173**, 32 (1928).

³² E. W. Balson, G. A. Earwicker, and A. Lawson, *Biochem. J.*, **29**, 2700 (1935).

discussion is necessary. However, there are other cases where the accuracy of the experimental procedure must be very high, but the meaning of pH is rather arbitrarily chosen for the particular case. There is no objection to such a practice, if the sole purpose of the procedure is to be reproducible in the laboratory. A good example of this kind is the pH value established for the fractionation and crystallization of proteins at $-5^{\circ}C$. in alcohol-water mixtures, by Cohn and associates.^{33, 34}

"The pH in these systems was controlled, wherever possible, by the use of buffers to yield the desired ionic strengths. The values of pH recorded do not refer to those in the ethanol-water mixtures at the low temperatures at which the proteins were fractionated. The pH was always determined by dilution of these systems to concentrations sufficiently low in ethanol that the pH value did not appear to change significantly with further dilution. In systems of low ionic strength, neutral salt solution was used as diluent. The measurements of pH were made with a glass electrode at a temperature near 25° ."

The idea underlying the choice of the above conditions is as follows. The difficulty in the definition of pH in partially alcoholic solutions is circumvented by diluting with water to such an extent that the effect of alcohol is practically negligible. The pH is not supposed to be essentially changed by the dilution, corresponding to the theory of buffers. By diluting the solution with a salt solution instead of pure water, or better still, to a definite ionic strength, say of $0.02 M NaCl$, the measurement is facilitated because of the higher conductivity of the salt solution, and the ionic strength of all solutions is the same during measurement. While this practice is adequate to give a reproducible procedure for the preparation of a compound, the meaning of pH in the particular case should always be carefully described.

V. OXIDATION-REDUCTION POTENTIALS

When an "indifferent" electrode of bright platinum or gold is immersed in a solution, even without bubbling with hydrogen, a potential is developed which is scarcely steady in time, nor is it reproducible at two or more such electrodes immersed in the solution.

Even if the solution contains no substance which is readily oxidized or reduced, some potential is established depending on the presence of some oxygen in the solution. When the oxygen is removed as much as possible by extended bubbling with purified nitrogen, a potential is established which is very much more negative than in the presence of oxygen, but which is

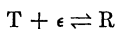
³³ E. J. Cohn, L. E. Strong, W. L. Hughes, Jr., D. J. Mulford, J. N. Ashworth, M. Melin, and H. L. Taylor, *J. Am. Chem. Soc.*, **68**, 459 (1946). The quotation is from page 463.

³⁴ E. J. Cohn, W. L. Hughes, Jr., and J. H. Weare, *J. Am. Chem. Soc.*, **69**, 1753 (1947).

neither well reproducible nor constant in time. It depends, to a large extent, on the fact that the metal occludes some oxygen which cannot be removed entirely at ordinary temperatures. The capacity of such a potential is poor. It can be easily shifted to a large extent by polarization even with weak currents. A reproducible, definite potential, which is not easily polarized, and after a slight polarization soon returns to its original value, is established only if the solution contains what is called a reversible oxidation-reduction system, or a "redox" system.^{35, 36}

A redox system is a mixture in a finite ratio of two molecular species which can be derived from each other by a reversible oxidation or reduction, as in a vat dye. In general, all measurements to be described here are carried out with homogeneous solutions. A good example of a redox system is, for our purpose, indigo disulfonate in mixture with its leuco dye. Indigo itself, although it is a vat dye, is too insoluble in water. Another good example is a mixture of potassium ferricyanide and potassium ferrocyanide. In the latter mixture, the oxidation or reduction is univalent; in the former, it is bivalent. The reduced form of a redox system may differ from the oxidized form either by one or more electrons, or by one or more hydrogen atoms (proton plus electron), the level of oxidation being judged only by the difference in the number of electrons, irrespective of the protons involved in the process.

Organic redox systems are usually bivalent. However, even a bivalent oxidation, provided it is reversible, appears to consist always of two successive steps of univalent oxidation.^{37, 38} Very often the two steps overlap so much that the redox process has the appearance, without a refined analysis, of being a single redox process in one, bivalent step. For a univalent redox system, the redox process may be formulated as:



where R is the reduced form,³⁹ T the oxidized form, e the electron, and the potential established at an indifferent electrode is (at 30° C.), in volts:⁴⁰

$$E = C + RT/F \ln [T]/[R] = C + 0.06 \log [T]/[R]$$

³⁵ W. M. Clark *et al.*, "Studies on Oxidation-Reduction," *U. S. Pub. Health Service Hyg. Lab. Bull.*, No. 151 (Feb., 1928).

³⁶ L. Michaelis, *Oxidation-Reductions Potentiale*, Springer, Berlin, 1928; 2nd ed., 1932. English ed., trans. by L. B. Flexner, Lippincott, Philadelphia, 1930.

³⁷ L. Michaelis and M. P. Schubert, *Chem. Revs.*, **22**, 437 (1938).

³⁸ L. Michaelis, *Ann. N. Y. Acad. Sci.*, **40**, 39 (1940).

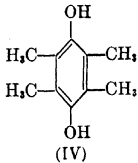
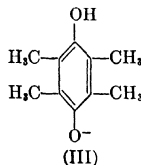
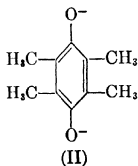
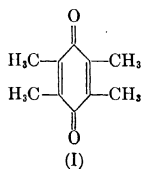
³⁹ Throughout, the oxidized (quinonoid) form of a reversible bivalent redox system is designed as T ("totally" oxidized form); from it there arises by a univalent reduction, the semioxidized form S, which is a free radical, and, by a bivalent reduction, the benzenoid or reduced form, or leuco dye R. In a univalent system, the symbol R is used for the reduced form, and T for the oxidized form.

⁴⁰ See also Chapter XXVIII, page 1791.

The brackets represent activities, which for all practical purposes may be taken as equal to the molar concentration, unless special conditions prevail and the experimental accuracy which is attainable justifies the consideration of activities instead of concentrations. C , a constant, is the potential if $[T] = [R]$, and is called the normal potential. If $[T]$ is either very small or very big compared with $[R]$, a small change in the concentration of the compound present in the low concentration causes a big change in E . If $[T]$ and $[R]$ are of the same order of magnitude, the dependence of E on their concentration is smaller and the system is "well poised." A large change in E is obviously characteristic for the end point (and the start) of a titration. Whenever R differs from T only by one or more electrons, the normal potential of the redox system is independent of pH . This is the case, for instance, in the system potassium ferricyanide + potassium ferrocyanide, except for extremely acid solutions. When, however, R differs from T by a hydrogen atom, the constant C depends on pH in such a way that C , plotted against pH , has a slope of 0.06 v. per pH unit. This value (0.06) holds for 29.5° C. and changes with the temperature, as is shown in table I (page 1730). In a bivalent redox system such as quinone + hydroquinone, where the overlapping of the two successive steps is so great that no intermediate univalent oxidation is noticeable within the limits of error, the factor RT/F must be replaced by $RT/2F$, or (at 29.5° C.):

$$E = C + 0.03 \log [T]/[R]$$

The following illustrate some of the cases that may occur. Among the quinonelike substances for instance, since benzoquinone is too unstable, especially in alkaline solution, duroquinone (tetramethylquinone) will be used as an example (I). In an extremely alkaline solution, in which the corresponding durohydroquinone (IV) is completely ionized to form II.



the normal potential is independent of pH . In a solution within such a pH range that only the univalent ion (III) appears (pH 12.5 to 14), C varies with pH by 0.03 v. per pH unit. In acid solution, where the neutral molecule (IV) exists, C varies by 0.06 v. per pH unit.

In a solution of sufficiently high alkalinity that only the bivalent ion (II) is present, C would be independent of pH , as stated above. However, it is impossible to prepare so alkaline a solution of the duroquinone system. Quite generally, only for very few organic redox systems can the condition be fulfilled that the normal potential is independent of pH . If at all, this happens only in an extremely high pH range, as can be generalized from the above example.

For comparatively few redox systems can a potentiometric measurement be carried out simply by dissolving a known amount of the T form and a known amount of the R form in a buffer solution and subjecting it directly to a measurement. The reason is that the reduced form in most systems is likely to be oxidized by the oxygen of the air before a measurement can be made. Among the few cases in which this difficulty does not arise to any noticeable extent are these systems: $FeCl_3 + FeCl_2$ in acid solution; $Fe(CN)_6K_3 + Fe(CN)_6K_4$ in not too strongly alkaline, or neutral, or slightly acid solution; or, quinone + hydroquinone in acid solution. The best way to carry out potentiometric measurements on redox systems is by potentiometric titration, either oxidative or reductive, in such a manner as to eliminate any trace of oxygen from the system, as is described below.

1. Reductive Titration. Reducing Agents

Usually the T form of a reversible redox system is stable in air, *e. g.*, methylene blue. In this case, a solution of T is made up in a buffer, the oxygen of the solution is thoroughly removed by bubbling with oxygen-free nitrogen, a reducing agent is added from a burette, in successive portions, and at each step the potential is measured. During the titration the solution is guarded against the access of any trace of oxygen.

The buffer should have an ionic strength, μ , not smaller, or at least not much smaller, than 0.1, and the concentration of the dye should be in the neighborhood of 10^{-3} *M*. If the concentration is too low ($\ll 10^{-4}$ *M*), the poise of the system may not be sufficient to establish reproducible potentials. If it is too high (10^{-2} or 10^{-1} *M*), the capacity of the buffer may not be sufficient to keep the pH constant during the titration if the T and R forms differ with respect to their acidic properties. The reducing agent must be in homogeneous solution and may be the R form of another reversible redox system of a much more negative normal potential than that of the system to be measured. Indophenol may be titrated with

leuco methylene blue, or, even better, with leuco rosindone sulfonate. Or, the reducing agent may be some irreversible system which performs the reduction practically instantaneously, in such a manner that its oxidation product does not interfere with the measurement because of any secondary chemical reaction. The concentration of the reducing agent depends on the purpose of the titration. This may be: (a) the determination of the amount of some oxidizable (or reducible) substance in the solution. Here, the titer of the agent used for titration must be exactly known, and the only aim of the titration is to ascertain the end point. Not all of the reagents mentioned below, especially among the reducing agents, are suitable for making up a standard solution of exactly known titer. Or it may be: (b) the determination of the normal potential and of some other characteristic properties of a redox system. Here, the titer of the oxidizing or reducing agent need be known only approximately. The end point of titration manifests itself by the jump of the potential, and it is the shape of the titration curve from which the wanted data are to be derived. The abscissa, originally plotted in terms of milliliters of the added reagent, is readjusted in terms of percentage of oxidation or reduction.

Out of the multitude of reducing or oxidizing agents, a few will be described which have proved to be especially convenient. Some other reagents may be found in the review by Furman.⁴¹

Sodium Dithionite, $\text{Na}_2\text{S}_2\text{O}_4$ ("Sodium Hydrosulfite").—Its application requires detailed directions. The solid substance, when dry, is perfectly stable in air. In solution, however, it is autoxidizable to an extremely high degree. It is not possible to keep a stock solution even approximately at its original titer. The solution must be prepared for immediate use. Even when kept protected from oxygen, it may gradually, sooner or later, undergo an irreversible decomposition, recognizable by turbidity caused by colloidal sulfur. This decomposition does not seem to occur in a slightly alkaline solution.

Dithionite is a very valuable and efficient reducing agent, yet its use is restricted. (a) It can be used only at $\text{pH} > 4$. (b) One must consider that, while acting as a reducing agent, it is oxidized to the bisulfite ion, HSO_3^- . This ion undergoes reactions with many compounds, *e. g.*, it combines with quinones, etc. Furthermore, bisulfite ion, or rather its nonionized form, namely, sulfurous acid, is itself a reducing agent. Although it is much less powerful than dithionite, and acts at a much slower rate, it may cause the potentials to drift in time. Dithionite can be used to titrate only dyes of such a negative potential range that sulfurous acid or bisulfite ion does not reduce them. Dyes with a normal potential decidedly more negative than methylene blue can be titrated, provided they form no addition compounds with bisulfite. Methylene blue itself cannot be titrated with dithionite (unless the titration is carried out within a few minutes) because it is reduced, at a slow rate, by sulfurous acid. Examples of dyes that can be successfully titrated with dithionite

⁴¹ N. H. Furman, *Ind. Eng. Chem., Anal. Ed.*, **14**, 367 (1942).

are riboflavin, rosindone sulfonate, and most of the phenazine derivatives, including the safranines.

Since the stability of sodium dithionite is improved by slightly alkaline reaction, its solution may be prepared in about 0.001 *M* sodium hydroxide. The buffer capacity of such a dilute sodium hydroxide solution is very small. Adding it to a well-buffered solution of great buffer capacity does not change the *pH* of the buffer to any appreciable extent. The following directions for making up a dithionite solution are therefore derived. A flask containing 20 to 30 ml. of water, after adding one drop of 0.05 *M* sodium hydroxide is closed with a stopper with two holes, one holding a glass tube as an inlet of pure nitrogen, the other as an outlet for the gases. A stream of nitrogen is bubbled for 15 minutes; then, while the gas flows, the stopper is lifted and a weighed amount of sodium dithionite is added; the stopper is reset, and the bubbling is continued for five more minutes. The glass tube, which has served as a gas outlet, is then pushed farther in so that the lower end is deep below the surface of the solution which, by the application of nitrogen pressure, is pushed into the burette previously washed with nitrogen in the manner described on page 1757. In estimating the amount of solid dithionite, it should be kept in mind that the commercial preparation is about 90% pure, that a small part may be lost by oxidation while the solution is prepared, and that one molecule of dithionite delivers two electrons (or two hydrogen atoms). The titer of the solution is known only to a rough approximation. For most purposes this does not matter, as can be seen later, and one can dispense with a determination of the titer.

Titanous Chloride.—The commercially obtainable solution of titanous chloride is made up in 15% hydrochloric acid and is rather stable in this condition. When diluted with water, so as to establish a less acid solution, it is easily oxidized in air. Therefore, any dilute solution must be immediately filled into the burette and protected from oxygen. Titanous chloride is a most useful reagent for reductive titration under the condition that the redox system is dissolved in a very acid solution, say ≤ 0.1 *N* hydrochloric or sulfuric acid; and the hydrochloric acid content of the dilute titanous chloride is so low that its addition to the redox solution does not change the *pH* to any appreciable extent. Because of its very negative potential range, titanous chloride is a useful and convenient reducing agent when applied to acid solutions. If one wishes to carry out a titration in extremely acid solution, say 5 *M* sulfuric acid, the stock solution of titanous chloride may be diluted, not with pure water, but with the same acid in such a concentration that the final concentration of the acid approximately matches that of the redox solution. In such a case, special attention must be paid to establishing constant liquid potentials (described on page 1768).

If one wants to use titanous chloride in less acid or even neutral solutions of the redox system, laborious procedures must be applied. Clark prepared solid titanous chloride, almost free of excessive hydrochloric acid, by evaporating the stock solution *in vacuo* and, protected from air, dissolving it in any desired weakly acid solution, or even in a citrate or tartrate buffer in which complex compounds are formed that are soluble even at rather high *pH*. The redox system may be dissolved in a similar buffer. In general, one may restrict the use of titanous chloride to very acid solutions, for which it is the most convenient reducing agent for titration.

Chromous Chloride.—Chromous chloride is prepared, according to Conant and Cutter⁴² by reduction of chromic chloride, using amalgamated zinc and hydrochloric acid. It is either kept over amalgamated zinc or is filtered through glass wool in an atmosphere of carbon dioxide. If the solution is filtered from suspended material and the acidity is not too high, it will keep for months without evolution of hydrogen.

If working with 1 to 10 *N* sulfuric acid (but not > 10 *N*) solution, chromous sulfate can be prepared by dissolving powdered chromium metal directly in the acid in a stream of hydrogen, with some heating if necessary. This solution can be directly pushed into the burette by nitrogen.

An approximately neutral, very poorly buffered, and, for this reason, convenient solution of a chromous salt can be made as follows. Chromoacetate is prepared according to Clark and Perkins⁴³ by reducing an aqueous solution of chromic chloride in hydrochloric acid with zinc dust. From the filtrate, crystalline, red chromoacetate is precipitated by addition of sodium acetate. The crystals are washed with water and dissolved in a large volume of air-free water. All manipulations are carried out in a stream of carbon dioxide. The final solution is washed with hydrogen and kept under hydrogen. The titer of such a solution is constant over weeks, *e. g.*, 0.0037 *N*.

Vanadous Chloride and Sulfate.—These compounds have been prepared by Conant and Cutter⁴² as follows: Vanadium pentoxide is reduced by amalgamated zinc and acid; although very much zinc is needed, the procedure is convenient and yields a solution containing a large amount of vanadous salt. As regards the range of usefulness of chromous and vanadous salts in their acid solutions, the same considerations hold as for titanous chloride.

Leuco Dyes.—These may be used as reducing agents provided their normal potential is decidedly more negative than that of the redox system to be titrated, so as to ascertain a distinct jump, or least inflection, of the potential curve at the end point of titration. For a number of cases, according to the normal potential of the redox system to be titrated, sodium indigo disulfate in its leuco form may be used. Rosindone sulfonate (rosinduline GG) in its leuco form is by far the most convenient "dye" to be used as a titrating agent. It can be used over a wide *pH* range and is stable even in very alkaline solution, a property shared by few other dyes of sufficiently negative potential and otherwise suitable properties as titrating agents. Rosindone sulfonate is not commercially available. Since it is almost indispensable for systematic redox studies its preparation is described in the following section.

Preparation of Rosindone Monosulfonate (Rosinduline GG).—A solution of 5 g. of rosindone trisulfonate (Rosinduline 2B, *National Aniline and Dye Co.*) in 60 ml. of water is heated in a sealed tube at 180° C. for 24 hours. Orange rosettes of free rosindone monosulfonic acid appear on cooling. The solution is acidified with 10 ml. of 2 *N* hydrochloric acid and the precipitate collected on the filter. If the starting material is good, the filtrate is practically colorless. The yield is 1.9 g. (70%). To convert the acid into the well-crystallized sodium salt it is suspended in 200 ml. of

⁴² J. B. Conant and H. B. Cutter, *J. Am. Chem. Soc.*, **48**, 1016 (1926).

⁴³ W. M. Clark and M. E. Perkins, *J. Am. Chem. Soc.*, **54**, 1230 (1932).

water and neutralized with sodium carbonate. Then 2 g. of sodium chloride is added and the solution heated to boiling. After 24 hours at room temperature, the product is filtered and washed with very little 1% sodium chloride solution. The compound should show at pH 4.64 the normal potential of -0.130 v., when the leuco dye is titrated with potassium ferricyanide, and an index potential (see below) of 15.1 mv.

The solution of the leuco dyes, either of rosindone sulfonate or of any other suitable dye, is made up as follows. A solution of the dye in adequate concentration (about 10 to 30 times that of the redox solution to be titrated) is made up, either in water or, if solubility properties permit, in the same buffer as is used as solvent for the redox system. However, since the volume change during the titration is small, preferably not more than 1/10, the pH of the buffered redox system is not changed beyond the limits of error even if the dye is dissolved in pure water. The latter is often preferable because most dyes are more soluble in pure water than in salt solutions. If the concentration of the redox system is $5 \times 10^{-4} M$, the concentration of the titrating leuco dye should be about $10^{-3} M$. The solubility of sodium rosindone sulfonate is about $2 \times 10^{-2} M$, except in very acid solution, in its yellow form, which is much less soluble. Therefore the restrictions imposed on the use of this dye because of its solubility properties are not serious.

The dye solution is filled into a bottle and closed with a two-hole stopper providing an inlet and outlet for gases. Then a small amount of colloidal palladium solution is added and hydrogen is bubbled through the solution until complete reduction of the dye is accomplished. For rosindone sulfonate, the color, originally red, turns on complete reduction to pale yellow or yellowish brown, according to the concentration. The amount of palladium solution depends on the specimen of colloidal palladium used. It is found, for instance, by adding one drop of a 1% aqueous solution to each 10 ml. of the dye solution. If, after 10 minutes of bubbling, the reduction does not proceed visibly, more should be added. The reduction may take, according to the concentration of the dye, from 10 to 30 minutes. It often starts but does not go to completion, the catalyst becoming inactive. If this happens, another drop should be added. The fact that the catalyst becomes inactive after a while is advantageous because it makes unnecessary the removal of the palladium before using the leuco dye for the titration. In any case the total amount of palladium should be so small that its gray color is not at all, or only slightly, noticeable in the leuco dye solution. When the reduction is complete, the hydrogen is displaced by nitrogen bubbled through the solution for a sufficient time, say 10 minutes, with five bubbles per second. The tube which served as gas outlet is pushed deep below the surface of the solution, and the solution is transferred by pressure of nitrogen into the burette previously washed with nitrogen, as described on pages 1763 *et seq.*

A modification of the method, used especially by Clark, is the addition of small pieces of platinum asbestos instead of colloidal palladium solution, and interposing of a glass wool filter between the solution and the burette in order to filter out the platinum asbestos. Even so, microscopic particles of colloidal platinum may be carried over. This is not important because the platinum catalyst also is usually inactivated at the time the solution is used.

The only disadvantage in the use of a leuco dye as a reducing agent lies in the fact that the color changes in the redox system during the titration are obscured. The normal potential of rosindone sulfonate is so negative that only rarely are occasions encountered when, in reversible redox systems, no recognizable end point of titration is established.

Ascorbic Acid.—Sometimes, at $pH < 6$, a freshly prepared solution of ascorbic acid may be successfully used as a reducing agent for systems with not too negative a normal potential.

2. Oxidative Titration. Oxidizing Agents

A compound in the reduced form can in many cases be titrated with an oxidizing agent. If a compound present in the oxidized form is to be titrated with an oxidizing agent, it is first reduced by a small amount of colloidal palladium and hydrogen gas. The completeness of reduction can be recognized by the change in color. It may be mentioned that, when bubbled long enough with hydrogen in the presence of colloidal palladium, a bright platinum electrode reaches a constant potential corresponding to that of the hydrogen electrode and that this potential can be used for the measurement of pH at the beginning of the titration. Then the hydrogen is driven out with pure nitrogen as thoroughly as possible. A check as to the completeness of the removal of hydrogen is the following. When the hydrogen potential is established and the bubbling with nitrogen is started, the potential gradually shifts toward the positive direction. The bubbling is kept on until the potential (which will not be well reproducible at two bright platinum electrodes) is about 100 mv. more negative than the normal potential of the system to be titrated. If the normal potential of the redox system is so negative that this condition cannot be fulfilled, one must use good judgment as to when to start the titration. During the titration, the behavior of the potential rise will show how well the starting time for the titration was chosen, and proper correction for the true zero point may be applied. The following discussion illustrates the manner of handling the situation.

Suppose the removal of hydrogen was practically complete before the first portion of the oxidizing agent was added. Then, even after the addition of the first few drops of the oxidizing agent, a constant and reproducible potential (at least within 1 mv. or so) will be established within about half a minute after the addition of the oxidizing agent. Further addition will cause the potential to rise and to be firmly established within a very short time, reproducible at different electrodes in the vessel to within 0.1 mv. and, further on, even better. Toward the end of the titration, the speed at which the potential is being established, and its reproducibility, may be lessened. This may happen when the oxidation is, *e. g.*, 95% complete.

In the very neighborhood of the end point, both the stability and the reproducibility of the potential will be much less satisfactory. However, on plotting the titration curve, one will see that this uncertainty has no significance as to the determination of the end point.

On the other hand, if the removal of hydrogen was not perfect, the potential at the beginning of the titration may not rise sufficiently, or even drift back in time. Then, before proceeding with the titration, the bubbling with nitrogen must be continued to remove the last traces of hydrogen, which may be only slowly released if a somewhat larger amount of colloidal palladium is present. On proceeding with the titration, the potentials will behave regularly, and from the plot of the titration curve the true zero point may be extrapolated. Although such an experience is not desirable, the experiment need not be discarded provided the extrapolation is small.

Bromine and Chlorine.—Solutions at any pH can be titrated with bromine and freshly prepared solutions can be easily standardized as to its titer, which is maintained over a sufficiently long time. Similarly, a freshly prepared aqueous solution of chlorine may be used.

Potassium Ferricyanide.—A freshly prepared solution of this reagent can be used over a wide pH range, excluding only extremely acid solutions. A similar reagent of still higher potential is $K_2Mo(CN)_6$, potassium molybdicyanide, described by Buchnall and Wardlaw.⁴⁴

Potassium Dichromate.—This compound can be used for acid solutions of $pH < 4$. At higher pH values, the oxidation is sluggish and may cause the potentials to be established with undue slowness.

Ceric Sulfate or Thallic Sulfate.—Ceric sulfate or thallic sulfate dissolved in sulfuric acid of suitable concentration can be used as oxidizing agents if work must be done in strongly acid solutions.

Benzoquinone.—Water is boiled to remove dissolved oxygen and, after cooling down to about 80° C., a proper amount of finely ground quinone is dissolved in it; the solution, still rather warm, is sucked into the burette. It is stable for a sufficiently long time, but gradually decomposes. It is not recommended for the titration of alkaline solutions because quinone is utterly unstable in alkali and may decompose before the added amount is thoroughly mixed with the solution. For acid solutions, it is a fairly convenient oxidizing agent. It has the advantage that no overoxidation will take place in such cases in which the T form of the redox system is likely to be irreversibly overoxidized with any appreciable speed. On the other hand, the various possible reactions which quinone may undergo make necessary good judgment in the use of this otherwise very useful reagent.

Dyes.—For redox systems of sufficiently negative potential range, the T forms of stable dyes of very positive range can be successfully used as oxidizing agents. The writer recommends especially the use of phenolindophenol, which is

⁴⁴ W. R. Buchnall and W. Wardlaw, *J. Chem. Soc.*, 1927, 2981.

easy to prepare⁴⁵ but not commercially available. Other, commercially available, dyes of the indophenol class should be properly tested on standard substances because the available specimens are rarely pure compounds.

Lead Tetracetate.—For solutions in water-free glacial acetic acid, a solution of lead tetracetate in glacial acetic acid can be used as oxidizing agent.⁴⁶

3. Apparatus for Redox Titration

For the measurement of potentials, the same instruments are used as for the determination of hydrogen-ion concentrations.

The electrode resembles the hydrogen electrode (page 1721), except for omitting the coating of platinum black. Whether the electrode should consist of bright platinum or gold has been the subject of much discussion. However, the difference in behavior is slight and there is no reason why the blank platinum electrode should not be used in routine work. It should be frequently cleaned in cleaning solution and in 10% sodium hydroxide, and thoroughly washed in water thereafter. A gold electrode is most easily prepared by coating bright platinum with gold. For this purpose, a platinum electrode is used as cathode, with another platinum electrode as anode, in a solution of 1% gold chloride and excess of potassium cyanide. Current is supplied from a 4-v. battery with proper resistance to allow a very slow deposit of gold. The coated electrode is then washed in nitric acid and water. The gold electrode is supposed to establish potential more quickly and more reliably than the platinum electrode in relatively weakly poised redox systems, but sometimes the opposite may be true, and when the system is well poised there should be no difference between the two types. Even mercury can be used as an indifferent electrode when the redox potential of the system is so negative that all traces of mercury ions, which may be formed at the surface of the mercury electrode⁴⁷ on immersing it in the solution, are reduced. This condition is fulfilled for all redox systems with a potential range more negative than that of the indophenol dyes. For phenolindophenol, for instance, mercury cannot be used as an electrode, but it can be used for indigo sulfonates. Since mercury does not absorb any hydrogen gas, the electrode vessel may even be bubbled with hydrogen instead of nitrogen. However, a mercury electrode is much less convenient to handle than a platinum electrode and will therefore not often be used.

It is necessary always to have at least two platinum electrodes in the titration vessel and to read the potentials of each for comparison. When

⁴⁵ W. Mansfield Clark, Barnett Cohen, M. X. Sullivan, H. D. Gibbs, and R. K. Canan, *U. S. Pub. Health Service Hyg. Lab. Bull.*, No. 151 (1928).

⁴⁶ L. Michaelis and S. Granick, *J. Am. Chem. Soc.*, **62**, 2241 (1940).

⁴⁷ L. Michaelis and H. Eagle, *J. Biol. Chem.*, **87**, 713 (1930).

the poise of the system is sufficient, that is, if the redox system is perfectly reversible and the ratio of the oxidized and the reduced forms of the dye is within the range of 10:1 to 1:10, the potentials should agree to at least 0.1 mv.; usually, they do better. In the neighborhood of the starting point and of the end point, the agreement may be less perfect. This may also happen in the neighborhood of the 50% point with a bivalent redox system in which the two successive steps are extremely widely separated and, consequently, the titration curve around the 50% point of titration is very steep. However, if the slope of the titration curve is extremely steep an uncertainty of the potential of a few millivolts has in general no bearing on the results obtained from the titration curve.

The electrode vessel, should, in general, be cylindrical, about 4-5 cm. in diameter and about 12 cm. high (Fig. 19). If feasible, 20-40 ml. of the solution to be titrated should be used, or at least enough to cover the electrode

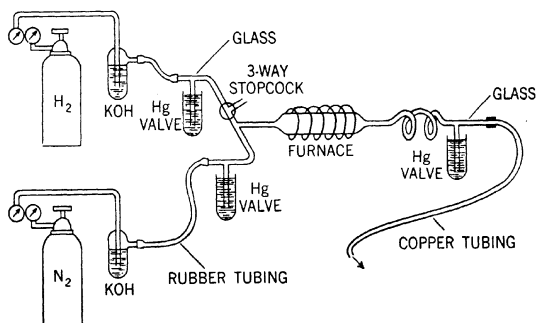


Fig. 17.—Train for the purification of hydrogen and nitrogen.

The glass tube after leaving the furnace should be slightly bent downward to avoid reflux of water of condensation into the furnace.

metals entirely. For special cases all dimensions may be increased or decreased. The upper end of the vessel is closed with a well-fitting rubber stopper with a number of holes through which glass tubes are tightly inserted. These tubes provide for two platinum electrodes (or even three), a gas inlet, a gas outlet, a KCl-agar bridge, a burette, and a reserve hole which may be closed or used for inserting a thermometer, or for the addition of a reagent, or for the third platinum electrode.

The gases used are pure nitrogen and, alternately, hydrogen. The latter is used when the solution, in the presence of palladium, is being reduced. Nitrogen is used to bubble out the hydrogen, or the oxygen of dissolved air, as the need may be, and is also used throughout the titration.

The speed of bubbling nitrogen should be great when oxygen or hydrogen is being removed (about 10 bubbles per second), and slow during the titration proper (about 3 bubbles per second).

Hydrogen and nitrogen are best supplied from cylinders with reduction valves. The gas train is shown in figure 17. From the cylinders, heavy-walled vacuum rubber tubes, which have been soaked for a day in about 5% sodium hydroxide solution and thoroughly washed with water, lead the gas to the purifying system in such a way that alternately the one or the other gas can be used. The gases pass first to a wash bottle with 10% sodium hydroxide, and then to a glass tube filled with copper filings, and are heated in the furnace to about 400° to 450° C. If care is taken that the temperature never exceeds 500°, Pyrex glass may be used. If no temperature control is applied, combustion-tube glass is necessary; however, it is more difficult to build up all glass parts with it. The writer has always used Pyrex glass, employing as a continuous measure of temperature a thermocouple of alumel-chromel, the soldered contact of the two metals being placed right above the Pyrex glass tube and covered by the lid of the furnace. The tube is filled either entirely with copper filings, freed from grease, or by alternate sections, each 10 cm. in length, of copper filings and tightly rolled copper gauze, which keeps the filings more firmly in place. After leaving the furnace, the gases should never be exposed to any rubber tubing which may give off volatile impurities and are never reliable with respect to impermeability to traces of oxygen. The end of the glass tube as it protrudes from the furnace is bent into a spiral for greater elasticity. The end is sealed to copper tubing of the flexible, elastic type, with DeKhotinsky, or a similar, cement. This copper tubing, of about 2-mm. inside diameter, leads to the gas inlet of the electrode. An airtight connection with the inlet tube may be obtained as follows. The upper end of the inlet tube is equipped with a glass bell (Figure 18) surrounding the open end. The protruding end has slipped over it a piece of rubber tubing, not too thin walled, exhibiting some rigidity and prepared as mentioned above, of such a length as to protrude from the opening of the glass tube, *e. g.*, 2 cm., into the bell. The end of the copper tubing is then pushed into this rubber tubing so as to be in contact with the end of the glass tubing, and the beaker is filled with mercury. The mercury is prevented from spattering by closing the beaker with a rubber stopper after first splitting the stopper into halves which can be properly put in place. The

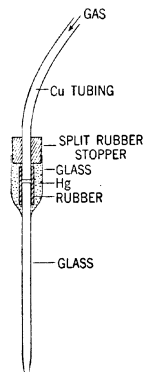


Fig. 18.—Joint between copper and glass tubing.

on continued bubbling. The gas may then be said, for all practical purposes, to be entirely free from oxygen.⁴⁹

Although the writer finds this simple procedure for purification of nitrogen combined with the possibility of the alternate use of nitrogen and hydrogen satisfactory in every respect, an alternate method used by Cameron⁵⁰ may be described here. Cameron purified and tested the nitrogen as follows. "Gas from the cylinder was passed through two towers of solid ammonium carbonate, and then through a tower of soda lime, leaving a definite partial pressure of ammonia in the gas. The mixture was passed through a quartz tube packed with platinized asbestos free from sulfur and heated to 450-500° C. Ammonia remaining after the reaction of ammonium and oxygen was removed with wash towers of 30 per cent sulfuric acid colored with bromophenol blue to indicate exhaustion, and acid spray and carbon dioxide were removed with a wash of 60 per cent potassium hydroxide. A bottle of a 0.1 per cent solution of reduced indigodisulfonate in the line indicated complete absence of oxygen, even at high rates of flow. It is important to insure saturation of the gas stream with ammonia, however. Exhaustion of any reagent was readily noted, and the line could be operated for long periods of time without attention."

The gas outlet is a glass tube, secured with a liquid seal. The agar bridge is the same as described previously. The end closed with a ground stopper is submerged in the solution, while the other end is dipped into a beaker containing a saturated solution of potassium chloride covered with a layer of white oil. Care should be taken that the end of the agar level is not, by accident, prevented by a drop of white oil from making contact with the solution. The tip of the calomel electrode is submerged in the same beaker of potassium chloride solution.

Figure 19 shows the type of burette recommended. The mixing of the reagent withdrawn from the burette may be accomplished by the bubbling gas, the speed of which may be temporarily increased for the purpose. Good judgment will show whether this procedure is sufficient. The writer prefers to agitate the vessel briefly (1 sec.) after each addition of reagent from the burette. Although this can be done by hand, a mechanical device is more convenient. In the writer's apparatus, the cylindrical vessel is held in a clamp, which is caused to oscillate about a perpendicular eccentric axis by a motor. The elasticity of the copper-tubing gas inlet permits the free movement of the vessel within the necessary limits.

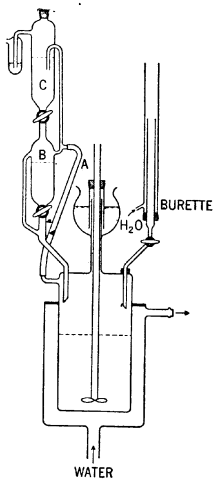


Fig. 20.—Cameron assembly for oxidimetric titration.

⁴⁹ H. von Wartenberg, *Z. Elektrochem.*, **36**, 295 (1930).

⁵⁰ A. E. Cameron, *J. Phys. Chem.*, **42**, 1217 (1938).

As regards temperature control, there is no objection to working at room temperature, provided the temperature is kept the same, within half a degree, in the calomel electrode and in the titration vessel. It is preferable, however, always to carry out the measurement at one temperature. The writer uses a compartment built in the laboratory and big enough for all manipulations, as an air thermostat at 30° C. Even so, the temperature both in the calomel electrode and in the titration vessel should be checked by a thermometer permanently enclosed in and protruding from the vessel. The potentiometer is outside the constant-temperature compartment. A water bath for temperature control can be used only with utmost precaution (see below), for the water film on the electrode walls is likely to cause stray currents. However, in Clark's laboratory, a thermostat with white oil, in which the electrodes are immersed, is successfully used.

A somewhat different apparatus used by Cameron⁵⁰ for the discontinuous titration of several organic developing agents is shown in its essential parts in figure 20. A double-walled beaker, the top of which is flattened and ground plane, is fitted with a flanged glass cap, also ground plane, carrying a water seal for the stirrer, a glass electrode, and a neck in which can be inserted a stopper carrying the electrodes. Lead-in tubes for the salt bridge and for the gas supply to a porous alundum bubble head are sealed through the cap. Three small necks on the cap permit insertion of burette tips with pieces of small rubber tubing for gasketing. A thermometer mounted in this same cap permits determining the reaction mixture temperature. Water is pumped through the jackets of the reaction vessel, the half-cell, and the burettes from a 35-gal. thermostat operating at 20° C. \pm 0.01°. The addition tube in figure 20 makes it possible to add the oxidizing solution rapidly and flush the vessel with buffer solution without admitting atmospheric oxygen. With the pinch clamp on rubber tubing *A*, nitrogen from the reaction vessel sweeps out compartment *B* containing the oxidizing solution, and bubbles through the buffer solution in compartment *C*, before escaping to the air through the bubbler. If the clamp is removed from the tubing, nitrogen from the reaction vessel can replace the solutions when the lower and upper stopcocks are opened in succession. The stopcocks are both of large enough bore to permit 10 ml. of solution to be added in about two seconds.

4. Measurements in Extremely Acid Solution

When a potentiometric titration is to be made on a solution which is much more acid than about 1 *N* HCl, the following considerations may be helpful. First of all, the necessity of more or less continuous bubbling with gas makes it unfeasible to use hydrochloric acid as an acid because of the evaporation of gaseous hydrogen chloride. However, sulfuric acid can be used up to a concentration of 10 *M* or more. The determination of *pH* with the hydrogen electrode is impossible, for not only is the liquid junction potential very large and of incalculable magnitude and very changeable in time, but the very definition of *pH* breaks down. However, a useful titration curve for a reversible redox system can be obtained⁵¹ as follows.

⁵¹ L. Michaelis, M. P. Schubert, and S. Granick, *J. Am. Chem. Soc.*, **62**, 204 (1940).

The closed tip of the KCl-agar bridge is immersed before use for many hours in an acid solution of the same composition as that of the experiment proper, while the other end is kept under a saturated potassium chloride solution. The acid is renewed several times. A quasistationary concentration gradient of the acid is gradually established within the agar tube. When the agar tube is now used for the titration, the liquid junction potential will be relatively stable. Although its magnitude is unknown and even not reproducible, it will be constant within approximately 1 mv. or so during the time necessary for the titration. The absolute values of the potentials have no significance, but the shape of the potential curve can be utilized for conclusions, as will be discussed presently. It may be mentioned that the titration curves obtained with certain bivalent redox systems in solutions of such high acidity can be used to make up a rational extension of the acidity scale, which may be regarded as an extension of the pH scale.⁵²

5. Measurement of pH in Redox Titration

All redox titrations must be carried out in well-buffered solutions which must fulfill two conditions. (1) They must possess a buffer capacity sufficient to keep the pH constant during the titration; and (2) the pH must be known either by using one of the standardized buffer solutions or, better, by direct pH measurement before or after the titration, or, when suitable, both before and after. Most of the usual buffers can be used. A few remarks will suffice.

(a) Borate buffer may cause erroneous results because of its inclination to form complex compounds, especially, but perhaps not exclusively, with molecules containing two adjacent hydroxyl groups. Indigo sulfonates, for example, seem to yield erroneous results when titrated in borate buffer.

(b) Glycine buffer cannot be used with quinones and probably with some other compounds because of chemical interaction. *N*-Dimethylglycine buffer⁵³ can be safely used instead. Its pH range is about 0.5 pH unit higher than that of glycine, the latter being around 9.5.

Buffers which may be recommended are those composed of a mixture of the following acids and their sodium or potassium salts: phosphoric acid either as a mixture of primary with secondary phosphates for the pH range from 5.5 to 7.5, or as a mixture of secondary and tertiary phosphates for pH from 10.5 to 11.3 or so, prepared by mixing *N*/15 disodium phosphate with sodium hydroxide in amounts so as not to convert the secondary entirely

⁵² L. Michaelis and S. Granick, *J. Am. Chem. Soc.*, **64**, 1861 (1942).

⁵³ L. Michaelis and M. P. Schubert, *J. Biol. Chem.*, **115**, 221 (1936).

into tertiary phosphate. Because of the large variation of pH in these buffers with change of ionic strength, the pH should be measured in the final mixture and tabulated values not relied upon. Acetate buffer can be safely used at pH from 5.8 to 3.4, a buffer of lactic acid and sodium lactate at pH from 4.5 to 3, and citrate buffer at pH from 3 to 1.5. For the pH range from 7.5 to 9.8 dimethyl barbiturate buffer,⁵⁴ prepared from sodium dimethyl barbiturate (sodium veronal) and hydrochloric acid is almost indispensable because of the deficiencies mentioned of other buffers in this pH range. For pH in the neighborhood of 1, hydrochloric acid can be used, *e. g.*, a solution of 0.1 *N* hydrochloric acid plus 0.5 *N* potassium chloride. For extremely acid solutions, outside the well-defined pH range, solutions of sulfuric acid are used, with the special precautions with respect to liquid junction which were described on page 1768.

The pH during the titration is measured as follows. In a *reductive* titration, after the redox titration is completed by titrating somewhat beyond the end point of reduction, some colloidal palladium solution is added. The bubbling with nitrogen is next replaced by bubbling with hydrogen, and the potentials are read until they stay constant in time. The pH is then calculated as for a regular hydrogen electrode. In an *oxidative* titration, the beginning of the experiment is usually the reduction of the solution with hydrogen and colloidal palladium. The reduction should be extended not only to complete reduction of the redox system, recognizable by the color change, but beyond, until a constant hydrogen potential is reached; from which the pH is calculated as usual. In these experiments, if desired, the pH can be measured once more at the end of titration, as a test of constancy during the titration, by adding a relatively large amount of colloidal palladium and bubbling with hydrogen until a constant hydrogen potential is reached. If it is desired to check the pH while the oxidimetric titration is going on (see below), a glass electrode may be used for this purpose. This electrode is standardized against the hydrogen electrode at the beginning or the end of the titration, or both.

6. Titration of Unstable Systems

If a component of a redox system is labile so as to undergo spontaneously some reaction other than that involved in the establishment of the redox equilibrium, no steady potential is obtained. Provided such a secondary reaction is sufficiently slow, the drifting potential may be extrapolated to a value corresponding to the initial state as it exists, or is imagined to exist, before the decomposition takes place. Continuous titration of such a system is impossible, but a discontinuous titration can sometimes

⁵⁴ L. Michaelis, *J. Biol. Chem.*, **87**, 33 (1930).

be helpful. For instance, to the solution of the stable reductant an amount of oxidizing agent is added which is estimated to produce a certain amount of the oxidant, which is unstable. The ratio of oxidant to reductant is fixed at least momentarily. The potential is read as quickly as possible and its drift in time is pursued. Extrapolation will give an approximate value of the original potential. Then another portion of the reductant is mixed with a larger portion of the oxidizing agent and the procedure repeated. When all the experiments are grouped together, a composite titration curve can be constructed and utilized as a regular titration curve, sometimes with good success, depending upon the lability of the system. Methods of "quick" titration, with or without extrapolation to zero time, have been used by Clark, Cohen, and Gibbs,⁵⁵ by Büllmann and Blum,⁵⁶ Fieser,⁵⁷ and others.

The same principle has been used by Ball and Chen⁵⁸ in a method based on a principle devised for other purposes by Hartridge and Roughton.⁵⁹ A solution of the reductant (for the details of this delicate procedure, the original literature must be consulted) under investigation and a solution of the oxidizing agent flow through a mixing chamber and then through a tube which at certain intervals is provided with platinum electrodes. If the mixing rate is high in comparison with the oxidation rate, the potentials along the tube change according to the rate at which the oxidation takes place. Each electrode shows a steady potential. With a stable system, such as Fe^{+++} - Fe^{++} , the potentials along the tube are alike. With a deteriorating system, the change in potential due to the deterioration is superimposed on the change caused by the oxidation process. The potential, then, can be extrapolated to zero time, and the rate of change gives the rate of the deteriorating reaction.

For another method of discontinuous titration with the apparatus (Fig. 20, page 1767), Cameron⁵⁰ may be quoted.

"The experimental procedure followed consisted in adding 100 ml. of buffer solution, 0.2 *M* in buffering ion and with a sodium-ion concentration fixed by addition of sodium sulfate, as necessary, to the reaction vessel, and determining the pH with two hydrogen electrodes. The glass electrode was then read, and the hydrogen displaced with pure nitrogen with the substitution of blank platinum and gold electrodes for the platinized ones. The addition tube was set in place with the

⁵⁵ W. M. Clark, B. Cohen, and H. D. Gibbs, *U. S. Pub. Health Service Hyg. Lab. Bull.*, No. 151, 245 (1928).

⁵⁶ E. Büllmann and J. Blum, *J. Chem. Soc.*, 125, 1719 (1930).

⁵⁷ L. F. Fieser, *J. Am. Chem. Soc.*, 52, 4915 (1930).

⁵⁸ G. Ball and T. T. Chen, *J. Biol. Chem.*, 102, 691 (1933). R. N. J. Saal, *Rec. trav. chim.*, 47, 73 (1928). H. Schmid, *Z. physik. Chem.*, A141, 51 (1929); 148, 321 (1930).

⁵⁹ A. H. Hartridge and F. J. W. Roughton, *Proc. Roy. Soc. London*, B104, 376 (1923).

measured amount of oxidizing solution and buffer solution for flushing, and half an hour allowed for the sweeping out of hydrogen and air. Ten milliliters of reducing agent was then added from one of the jacketed burets. The lower stopcock of the addition tube was opened, and at the same time an electric timer was started which gave a single stroke on a bell at 15-sec. intervals. The upper stopcock was then opened and the lower chamber flushed into the reaction vessel.

"Readings were taken at 15-sec. intervals for 3 min. and at 1-min. intervals for 7 min. The glass electrode was then read again, and the pH of the solution as originally determined with the hydrogen electrode was corrected for any change in pH which had occurred. Readings were taken with a student-type potentiometer. The potential of the glass electrode was read with the same potentiometer, using a ballistic galvanometer and a 1 microfarad condenser as null point indicator.

"Addition of the solutions raised the temperature of the reaction mixture slightly, but this effect could be disregarded, since in most cases the extrapolation to zero time was made from a graph which was a straight line up to 10 min."

7. Interpretation of Titration Curves

To interpret the titration, the potential is plotted against the volume of the titrating agent. At the end point, the potential changes abruptly (see

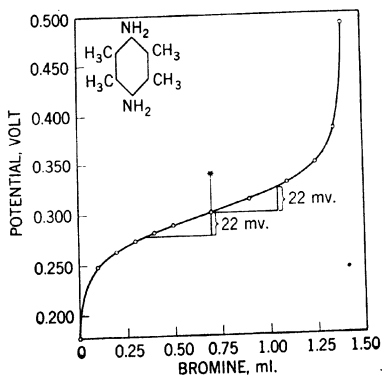


Fig. 21.—Titration of the *p*-diamino-durene system.⁶⁰

fig. 21), and, if the titer of the oxidizing (or reducing) agent used for the titration is known, the amount of redox system in question can be determined. Even without accurate knowledge of this titer, many data can be derived from the titration curve. First of all, the symmetry of the curve around its mid point (at 50% oxidation) should be checked. The potentials from 0 to 5% and those from 95 to 100% are not very reliable in this

⁶⁰ L. Michaelis, M. P. Schubert, and S. Granick, *J. Am. Chem. Soc.*, **61**, 1981 (1939).

respect. In more difficult cases, *i. e.*, at very low concentration of the redox system or when the substance to be titrated is contaminated with some other oxidizable or reducible material, the potential ranges of which overlap somewhat with the redox system proper, the unreliable range may extend to 10% and from 90%, respectively. The best method for first orientation is to check whether the difference in potential, the so-called index potential, E_i , is, say, within 0.2 mv., the same, no matter whether it is taken as the difference at the 50% and 25% points of the titration, or at the 75% and 50% points. For instance, in figure 21, E_i is 22 mv.

A. The titration curve is symmetrical. Then:

(1) When E_i is 28.6 mv. (the limit of error may usually be regarded as ± 0.2 mv.), with corresponding values chosen for other temperatures in proportion to the values of table I (page 1730), the redox system is a univalent one. In exceptional cases, recognizable by the fact that during the titration a twofold change in color takes place, it is a bivalent redox system with a semiquinone formation constant = 4 (see below). Such cases will never cause any doubt because this event can occur, for any one redox system, only at one particular pH. The same dye at any other pH will show an E_i different from 28.6 mv.

(2) When E_i is 14.3 (± 0.2) mv. at 30° C., the redox system is a bivalent one, without the formation to any measurable extent, of any intermediate step by univalent oxidation.

(3) When the index potential, E_i , in a redox system previously known to be bivalent, is > 14.3 (± 0.2) mv., there exists an intermediate univalent step of oxidation. If, on varying the initial concentration of the redox system (say, from 5×10^{-4} to 3×10^{-3} M) in the same buffer, E_i is unchanged, the intermediate step of oxidation is a free semiquinone radical. On the other hand, if E_i depends on the initial concentration, the intermediate step is not only represented by this free radical but also by a dimerization product of the radical with which it is in equilibrium.

(4) When E_i is ≥ 40 mv., no other point of inflection should appear than that at 50% oxidation. Whenever E_i is > 40 mv., in addition to the point at 50% oxidation, two lateral points of inflection appear. This demonstrates even at first inspection that the oxidation proceeds in two successive steps. When E_i is extremely large, say $\gg 200$ mv., the two lateral points of inflection lie at 25% and at 75% oxidation. In such a case, the first half of the curve (from 0 to 50%) may be considered as representing a univalent oxidation which, considered separately, has the $E_i = 28.6$ mv.; and the same holds for the second half, from 50 to 100%.

(5) Let us define the semiquinone formation constant, k , as follows:

$$k = s^2/rt$$

where s is the concentration of the semiquinone radical, r , that of the reduced form, and t , that of the (totally) oxidized form of the redox system. One derives k from E_i , as follows:

$$\sqrt{k} = 10^{E_i/0.06} - 3 \times 10^{-E_i/0.06}$$

where E_0 , the absolute value of the index potential in volts, is always taken as a positive number. (The factor 0.06 holds for 30° C. Compare table I, page 1730.) The maximum amount of the semiquinone radical exists at 50% of oxidation, and the ratio of the semiquinone to the total dye, $(s/a)_{\max.}$, at that point is:

$$(s/a)_{\max.} = \sqrt{k}/(2 + \sqrt{k})$$

(6) The normal potential of the bivalent system R,T or the mean normal potential, E_m , is the potential at 50% oxidation.

(7) The normal potential of the lower step of oxidation, E_1 , or the normal potential of the R,S system is:

$$E_1 = E_m - 0.03 \log k$$

where k is the semiquinone formation constant, and E_m the potential at 50% of the oxidation. The normal potential of the higher step of oxidation E_2 , or the normal potential of the S,T system, is:

$$E_2 = E_m + 0.03 \log k$$

and always we have:

$$E_m = (E_1 + E_2)/2$$

E_m may be interpreted as the mean normal potential, or the normal potential of the system considered as a bivalent redox system. E_1 is the normal potential of the lower (univalent) step of the oxidation, E_2 that of the higher (likewise univalent) step.

B. The titration curve is not symmetrical about the point of 50% oxidation and/or the shape of the curve is not independent of the concentration of the redox system:

(1) The curve is symmetric but its slope varies with the concentration. The intermediate step of oxidation is not represented by a free radical, S, alone, of the same molecular size as T or R, but by a dimerization product of the radical in equilibrium with the free radical. This is true, for instance, for phenanthrene quinone-3-sulfonate in alkaline solution. On lowering the concentration, one may reach a concentration range in which the titration curve no longer depends on the concentration. In this range of concentration, the dissociation of the dimer into free radicals is practically complete, and then the mathematical analysis of the limiting curve for extremely high dilution is the same as before. From the deviation at higher concentration, the constant of dimerization can be calculated. As to the details of such calculations, the original papers may be consulted.⁶¹

One single case has been encountered thus far, namely, phenanthrene quinone sulfonate in acid solution, in which in an homogeneous solution an intermediate step of oxidation is formed consisting practically entirely of the dimerized form of the radical. Here, at very low concentration, no intermediate step is formed in a

⁶¹ L. Michaelis and M. P. Schubert, *Chem. Revs.*, **22**, 437 (1938). L. Michaelis and E. S. Fetcher, *J. Am. Chem. Soc.*, **59**, 2460 (1937). L. Michaelis and G. Schwarzenbach, *J. Biol. Chem.*, **123**, 527 (1938).

distinctly measurable amount, the curve having an index potential of 14.3 mv. within the limits of experimental error; but in higher concentration the slope of the titration curve becomes increasingly dependent on the concentration, and the formation of an intermediate step of oxidation can be recognized also by the twofold color change during the titration.

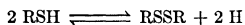
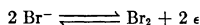
(2) The titration curve is not symmetrical around its 50% point. In this case, the molecular size of the T form differs from that of the R form. In many dyes, the T form, at not too low a concentration, has the tendency to form polymers which, at not too high a concentration, are usually dimers.⁶³ The R forms (leuco dyes) are not known to have a comparable tendency to polymerize in solution.⁶²

In the concentration range from 10^{-3} to 10^{-4} M, this phenomenon has no conspicuous effect on the shape of the titration curve. It is just noticeable, for instance, with methylene blue. In higher concentrations, the phenomenon can be studied by spectrophotometric methods, but not very well potentiometrically because, as stated above, it is hardly possible to keep the pH constant during a titration unless the concentration of the dye is very much smaller than that of the buffer.

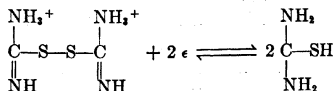
A special case of titration curve which is neither symmetrical about its midpoint nor independent of the initial concentration of the substance to be titrated is encountered if the chemical process takes place according to equations (1) or (1a):



Two examples are:



where RSH is any sulfhydryl compound such as thioglycolic acid, and RSSR is the corresponding disulfide. However, most of the sulfhydryl compounds do not form truly reversible redox systems. Recently Preisler⁶⁴ discovered reversible sulfhydryl systems which establish reproducible potentials as postulated theoretically. The first case of this kind is the reversible oxidation of thiourea to formamidine. Thiourea does not ionize to any appreciable extent, whereas formamidine is a bivalent base. The reaction is therefore:

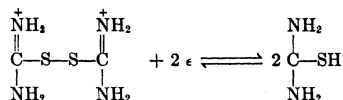


⁶³ It may be noted that, according to G. N. Lewis and J. Bigeleisen, *J. Am. Chem. Soc.*, **65**, 2419 (1943), leuco methylene blue is dimeric at the temperature of liquid air in a rigid solvent. There is no evidence of dimerization at room temperature.

⁶⁴ E. Rabinowitch and L. F. Epstein, *J. Am. Chem. Soc.*, **63**, 69 (1941).

⁶⁵ P. W. Preisler and L. Berger, *J. Am. Chem. Soc.*, **69**, 322 (1947).

The writer wonders whether this reaction should be formulated as follows:



This formula would permit a symmetric resonance structure of the formamidine ion and would account for its greater stability in acid solution.

On studying the reaction within a pH range in which change of pH does not alter the ionic state of formamidine, *e. g.*, in hydrochloric acid of various concentrations, the electrode equation is:

$$E = E' + 0.03 \ln \frac{[\text{RSSR}]}{[\text{RSH}]^2}$$

where the constant E' may be considered as the "normal potential," namely, the potential under the condition that the logarithmic term vanishes. Obviously, this does not happen either when the ratio of the concentration of the oxidized and the reduced forms is 1:1, or at any other fixed value of this ratio, but rather this "normal potential" depends on the absolute concentration. The titration curve is not symmetrical about its midpoint at 50%; the point of inflection lies at that point where the ratio of the molar concentration of the reduced form to the (equivalent) concentration of the oxidized form equals $2 - \sqrt{2}$, or at 41.4% oxidation. This asymmetry is not very striking on inspection of the graph. Much more striking is the fact that the shape of curve, and also the "normal potential," depends on the initial concentration of the substance. The dependence of potentials on the initial concentration is a very much more sensitive criterion for the existence of this type of redox system than the asymmetry of the titration curve. It should be mentioned that Preisler⁶⁵ has also shown the existence of truly reversible titratable redox systems of a sulfhydryl compound with a symmetrical titration curve, such as dithiobiuret-dihydrodithiobiuret.

It should be added that the theory of reversible two-step oxidation can be extended, with a slight modification, to another type of system. In the preceding section, the reversibility was restricted to a bivalent oxidation, which could be separated into two successive univalent steps. Usually, the reversibility does not extend over a wider range than the two-step oxidation. However, some cases have been described where a reversible bivalent oxidation can be extended to a further reversible bivalent oxidation. The first case to fulfill this condition at least approximately concerned a naturally occurring vegetable pigment from the plant *Mercurialis perennis*.⁶⁶ A carefully investigated case⁶⁷ is the oxidation of hexahydroxybenzene to tetrahydroxyquinone, and further on to rhodizonic acid. Each of these two steps is reversible and is a bivalent oxidation which, under the condi-

⁶⁵ P. W. Preisler and M. M. Bateman, *J. Am. Chem. Soc.*, **69**, 2632 (1947).

⁶⁶ R. K. Cannon, *Biochem. J.*, **20**, 927 (1926).

⁶⁷ P. W. Preisler, L. Berger, and E. S. Hill, *J. Am. Chem. Soc.*, **69**, 326 (1947).

tions studied, does not give rise to any appreciable amount of semiquinone. Such a case can be dealt with in the same way as before with the exception that each time where the factor RT/F was formerly used in the underlying theory, the factor $RT/2F$ must be applied. Here also, one can distinguish the normal potential of the lower step, that of the higher step, and the mean normal potential of the whole system. The plot of such normal potentials resembles in principle that of the example shown in figure 24.

8. Enzymatically Activated Redox Systems

Many cases have been discovered in biochemistry in which two molecular species, differing only in the level of oxidation but not behaving as reversible redox systems, become reversible systems upon addition of an enzyme. Nevertheless, no metal electrode responds to such an enzyme-activated system so as to establish a redox potential. These systems, however, can be brought into equilibrium with a regular redox system in the solution. The latter will then impress its potential on the electrode and so permit an indirect measurement of the state of the other system in terms of a redox potential. The principle of those measurements is the same in all cases; and the first case discovered, by Thunberg⁶⁸ and Quastel,⁶⁹ may therefore be sufficient to illustrate the experimental procedure.

Fumaric acid and succinic acid differ only by two hydrogen atoms. A mixture of the two, by the addition of an enzyme, can be said to form a reversible redox system. The substance used as an enzyme is succinodihydrogenase, extracted from muscle tissue. On adding a small amount of methylene blue to the substrate-enzyme system, methylene blue is partially reduced to such an extent that an equilibrium between the substrate and the dye system is established. Provided the concentration of the dye is very much smaller than that of the organic acids, the redox state of the dye is just an indicator for that of the organic acids. The reaction between the organic acid system and the dye is slow, and the establishment of the definite potential requires considerable time, some minutes to an hour.

The dye used as indicator must have a normal potential not too different from that of the inert system. Only if in the final state of equilibrium the concentration of the oxidized and the reduced form of the indicator dye is of the same order of magnitude is the poise of the dye system sufficient to establish a reproducible potential of thermodynamic significance. In order to test the reversibility of the system, one has to carry out measurements with varied ratios of the two acids to show that the variation of the potential is that expected for a reversible system.

⁶⁸ T. Thunberg, *Skand. Arch. Physiol.*, **46**, 339 (1925).

⁶⁹ J. H. Quastel and M. D. Whetham, *Biochem. J.*, **18**, 519 (1924).

A number of dye indicators which may be used for different potential ranges at pH 7 are given in table IV.

TABLE IV
SOME RECOMMENDED REDOX-INDICATOR DYES

Dye	Normal potential at pH 7, v.
<i>m</i> -Bromophenolindophenol ^a	+0.25
Thionine (Lauth's violet) ^a	+0.13
Methylene blue ^a	+0.01
Indigo tetrasulfonate ^a	-0.05
Indigo disulfonate ^a	-0.12
Rosindone sulfonate (Rosinduline GG) ^b	-0.28
Benzylviologen ^c	-0.36
Methylviologen ^c	-0.45

^a W. M. Clark, *The Determination of Hydrogen Ions*. 3rd ed., Williams & Wilkins, Baltimore, 1928.

^b L. Michaelis, *J. Biol. Chem.*, **91**, 369 (1931).

^c L. Michaelis and E. S. Hill, *J. Gen. Physiol.*, **16**, 859 (1933).

9. Quasireversible and Irreversible Systems

As mentioned above, it may happen that, in a redox system, one of the forms is not a stable molecular species but gradually undergoes an irreversible change. Sometimes it is the reduced form, R, and sometimes the T form which is labile. The writer has never encountered a system with stable or even moderately stable R and T forms in which the semiquinone radical, S, is conspicuously labile so as to undergo an irreversible decomposition more rapidly than the R or T forms. According to the velocity of such a secondary irreversible reaction, a potentiometric oxidative or reductive titration can be executed with fair accuracy provided the whole titration is performed within a short time. Samples of redox systems with a labile R form are the indigo sulfonates in alkaline solution (pH 11-12). Examples with a labile T form are the aromatic *p*-diamines, of which the T form is the diimine, which are so labile in aqueous solution that no reliable end point can be obtained. In some of these cases,⁷⁰ namely, if the two steps of the bivalent oxidation are sufficiently separated so as not to overlap too much, the end point of the first step of oxidation can be obtained more or less satisfactorily. Any irreversible decomposition of one of the forms of the system causes the potential to drift. If the drift is small, say 0.2 mv. per min., and the titration can be finished within five minutes, a rather useful titration curve can be obtained and the required data can be derived from it, like the normal potential, the semiquinone formation constant, etc.

⁷⁰ L. Michaelis, M. P. Schubert, and S. Granick, *J. Am. Chem. Soc.*, **61**, 1981 (1939).

The more rapid the decomposition, the less satisfactory is the titration curve and, in extreme cases, which only too often occur, it may be quite useless. Such systems are transitions to irreversible systems. Special techniques for labile systems were mentioned on pages 1102 and 1103.

Among the irreversible systems, two types can be distinguished, although transitional cases may occur. In the first class, oxidation-reduction can be accomplished in a reversible way, speaking purely chemically but not thermodynamically. For instance, the di- and triphenylmethane dyes can be completely reduced in a slightly acid solution, *e. g.*, by zinc dust, and re-oxidized with 100% yield, *e. g.*, by oxygen with a trace of a ferric salt as catalyst. Reduction is possible only with a very powerful reducing agent, and reoxidation only by a very powerful oxidizing agent, probably because of the fact that the semiquinone formation constant is extremely small, so that the concentration of the semiquinone, which is necessary for the progress of the bivalent over-all oxidation, is, under all conditions, vanishingly small. A transitional case is that of duroquinone (dissolved in 20% pyridine + 80% water, for better solubility). This system can be titrated perfectly well from pH 14 to 6, but at $\text{pH} < 6$ the establishment of the potentials becomes sluggish and the results are not strictly reproducible.

In the other type of irreversible system, either the oxidation or the reduction can be brought about only by strong agents, often only in a round-about way, and the yield on reversing the process, if any, is small. Such a system is encountered when an azo dye is reduced in acid solution, where the first stable reduction product results from the irreversible splitting of the $\text{N}=\text{N}$ group into two NH_2 groups. Here, there is no meaning in a "potential" in the thermodynamic sense.

Conant and Fieser⁷¹ have introduced the terms "apparent oxidation potential" and "apparent reduction potential." The "apparent potential" is the potential of a reversible redox system which is just able to bring about the oxidation (or reduction) of the irreversible system at a noticeable rate. The speed of the reaction can be observed by watching the drift of the potential of the reversible agent when acting upon the irreversible one. Nitro compounds belong to this group of irreversibly reducible substances.

Finally a class of redox systems of very doubtful reversibility should be mentioned, the sulfhydryl compounds (mercaptans), such as thioglycolic acid or cysteine. In a purely chemical sense, the reaction, $2 \text{RSH} \rightleftharpoons \text{RSSR} + 2\text{H}$, can be reversed, but not thermodynamically. The chemical cycle can be completed only with expenditure of large amounts of energy. Mixtures of the oxidized and the reduced form establish very sluggishly

⁷¹ J. B. Conant and L. F. Fieser, *J. Am. Chem. Soc.*, **45**, 2194 (1923).

a not very well reproducible potential. Within the large errors involved when results are not reproducible, this potential seems to be independent of the concentration of the oxidized form. Although several authors have claimed the attainment of reproducible and thermodynamically significant potentials, there was no generally accepted confirmation of these findings. Here it is obviously not true that the reaction of these substances is only the withdrawal from, or the transfer to, the metal electrode, of electrons, but other reactions occur also, namely, the formation of complex compounds with the metal, at least at its surface.

However, recently Rykkan and Schmidt⁷² have claimed that cysteine, dissolved in potassium iodide solution, can be titrated with iodine, and that the potentiometric titration curve obtained thereby agrees with that expected for a reversible cysteine-cystine system. Probably the reversible iodide-iodine system acts as a kind of catalyst for the establishment of the cystine-cysteine potential in the same manner as the redox dyes act as catalysts in the reactions mentioned above. Many other sulphydryl compounds respond to this method in the same manner, according to the claim of these authors which, however, so far have not yet been corroborated by other authors. Concerning the truly reversible sulphydryl systems see above.^{64, 65}

10. Examples of Titration Curves and Potential-pH Curves

For an example of a bivalent reversible titration curve with $E_i = 22$ mv., we refer to figure 21 (page 1772). Here, a 0.001 *M* solution of diaminodurene, dissolved as its hydrochloride in 8 volumes of methanol + 2 volumes of aqueous acetate buffer of pH 4.64 (apparent pH of the mixture is 6.21) is titrated with bromine dissolved in the same solvent. The curve is symmetric and its shape is independent of the initial concentration (provided it is low—in high concentrations a partial dimerization of the radical occurs). The index potential is 22 mv., hence the semiquinone formation constant k is 1.0 and the ratio of semiquinone to total substance is 0.33 at 50% oxidation. The color turns, during the titration, from colorless to yellow to colorless. The T form, durene diimine, is, in this particular solvent, just stable enough to allow a titration experiment without undergoing irreversible changes to any noticeable extent. The mean normal potential $E_m = 0.300$ v. The potentials of the (univalent) lower, and that of the higher step of oxidation, are $0.300 \pm 0.06 \times \log 1 = 0.300$, hence $E_1 = E_2 = E_m$ for this particular case, where $k = 1.0$.

Figure 22 presents Cameron's results⁷³ on the quinone-hydroquinone

⁷² L. R. Rykkan and C. L. A. Schmidt, *Univ. Calif. Pub. Physiology*, **8**, 257 (1944).

⁷³ A. E. Cameron, *J. Phys. Chem.*, **42**, 1217 (1938).

system. How potentials vary with the pH of the solution if the reduced and oxidized forms differ only not by electrons but by hydrogen atoms was explained on page 1755. Such potentials may be considered as determined by the concentrations of the oxidized form and the *ion* of the reduced form. Then, for the quinone-hydroquinone system, the following equation results:

$$E = E_0 + \frac{RT}{2F} \ln \frac{(C_6H_4O_2)}{(C_6H_4O_2^{--})} = E_0 + \frac{RT}{2F} \ln \frac{(\text{quinone})}{(\text{hydroquinone})^*} + \frac{RT}{2F} \ln \frac{K_1 K_2 + K_1(H^+) + (H^+)^2}{K_1 K_2}$$

K_1 and K_2 are the first and second dissociation constants of the hydroquinone. The importance attached to the divalent ion corresponds to the

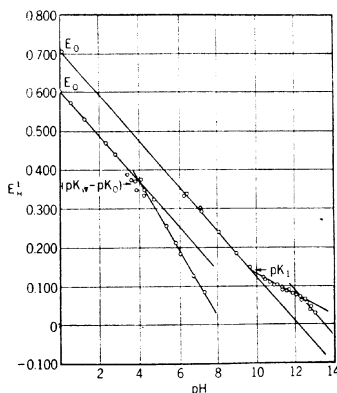


Fig. 22.—Normal potentials of the hydroquinone system at different pH values.

fact that neither the undissociated hydroquinone nor the monovalent ion forms quinone simply by losing electrons, while the difference between divalent ion and quinone consists of two electrons only. This provides a convenient picture of the processes involved, but it should be remembered that the thermodynamic results are independent of the mechanism by which they are reached.

The potential falls rapidly with increasing alkalinity of the solution. When $[T] = [R]$, the second member of the equation becomes zero, and at 20°C . the slope for the dependence of the redox potential upon pH

* Analytical concentration.

should be 0.058 until the pK of the first ionization constant is reached, *i. e.*, the pH at which the concentration of the monovalent ion equals that of the undissociated hydroquinone or; roughly, the pH at which 50% of the hydroquinone is ionized. Then the slope should become 0.029, and, at the pK of the second ionization, it should become zero. Examination

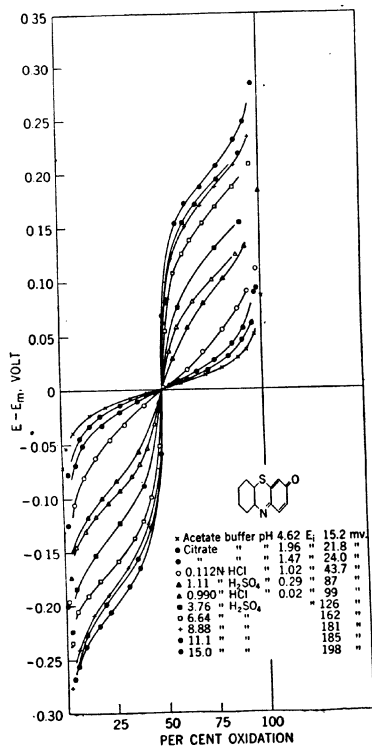


Fig. 23.—Titration of oxythiazine at different pH values.⁷⁴

of figure 22 shows that the change at the pK of the first ionization occurs, but that at about the point at which the pK of the second ionization should be, the slope reverts to the original 0.058. Furthermore, the slope between pH 9.8 and pH 12.3 is not the theoretical 0.029, but 0.0261. The irregular

⁷⁴ S. Granick, L. Michaelis, and M. P. Schubert, *J. Am. Chem. Soc.*, **62**, 1802 (1940).

slope indicates that in this region the potential is determined by a mixture of two systems, for the analysis of which the original paper should be consulted.⁵⁰ Similar considerations can be applied to other systems. This example is given just as a warning that, on working with substances susceptible to irreversible changes in alkaline solutions, as quinone, one cannot expect the anticipated results with neglect of such irreversible changes.

Figure 23 shows the variation in shape of the titration curve with change of acidity for a particularly suitable dye, *viz.*, oxythiazine.⁷⁴ The potentials are plotted, not referred to the normal hydrogen electrode as usual, but referred to the normal potential of the dye system at each particular pH. It can be seen that all curves with index potential $E_i < 40$ mv. have, only one point of inflection, but all those with $E_i > 40$ mv. have two more,

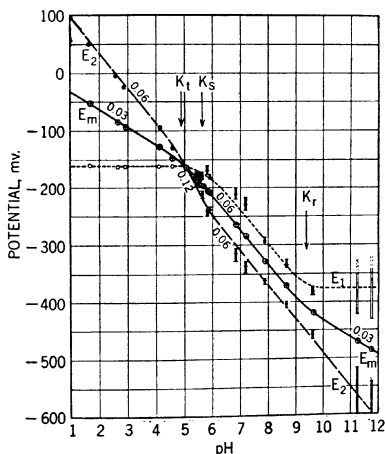


Fig. 24.—Normal potentials of pyocyanine at different pH values.⁷⁵

lateral points of inflection, which in the extreme case lie at 25 and 75% oxidation and thus separate the whole curve into two successive curves, each characteristic of a univalent oxidation.

In figure 24, the three normal potentials, E_m , E_1 , and E_2 for pyocyanine (*N*-methyl α -hydroxyphenazine) are plotted against pH. At pH = 4 there is a crossing point of the three curves. Here the semiquinone formation constant, k , equals 1, and $E_m = E_1 = E_2 = 1$, and the maximum

⁷⁵ L. Michaelis, E. S. Hill, and M. P. Schubert, *Biochem. Z.*, 255, 66 (1932).

ratio of the amount of semiquinone to total dye, $(s/a)_{\max.}$, equals 0.333. To the left of this crossing point, at higher acidities, the curves diverge, and $E_2 > E_m > E_1$. To the right of the crossing point, there is the reversed order, $E_2 < E_m < E_1$, and $k < 1$. In this region, all calculations depend on very slight variations of E_i and have relatively large limits of error, which are shown in the graph on the assumption that the index potential may be uncertain to ± 0.2 mv. The bends of the curves correspond to the acidic ionization constants of the reduced, semioxidized, and totally oxidized form, K_r , K_s , and K_o , respectively.

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POLAROGRAPHY

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I. INTRODUCTION

The preparation of polarograms or other polarographic data requires little skill and can be largely automatic. The simplest or empirical method, in which results are compared with previously constructed calibration curves, is quite adequate for routine analyses, provided that these have been expertly designed and that the unknown solutions are analyzed under the same experimental conditions as the standards. However, for the understanding of unforeseen complications and for the development of new and different procedures, a thorough knowledge of polarography is necessary. Consequently this chapter is not intended to describe available analytical procedures in detail; instead, it is designed to instruct the organic chemist sufficiently in these essentials that he may develop his own analytical procedure.

Polarography is an electrochemical method of analysis, incorporating features of potentiometry and electrolysis, but distinct from both. In polarography, *potentials are measured while known currents are flowing* through a cell in which one electrode is a dropping mercury electrode or other microelectrode. The currents are usually small and the electrolysis is of short duration; hence, the body of the solution remains practically unaltered. By plotting current against the corresponding potentials, one obtains a curve which, under suitable conditions, indicates the nature and concentration of the *reacting*, so called *electroactive*, material or materials present. Simultaneous qualitative and quantitative analyses of one or more compounds are thus possible.

The polarographic method of analysis was invented in 1922 by J. Heyrovský of Prague, and its development during the first 15 years was almost the exclusive work of this investigator and his collaborators. Polarography resulted from studies of electrolyses with a slowly dropping mercury electrode. This type of electrode had been introduced in 1903 by Kučera,¹

¹ G. Kučera, *Ann. Physik.* 11, 529 (1903).

who used the weight of the mercury drops for calculating the surface tension of mercury under different conditions. Heyrovský² made this electrode part of a cell to which he applied different voltages. Plotting the resulting current against the applied voltage, he obtained current-voltage curves with unique characteristics which could be used analytically. These curves showed great reproducibility because of the regularity with which

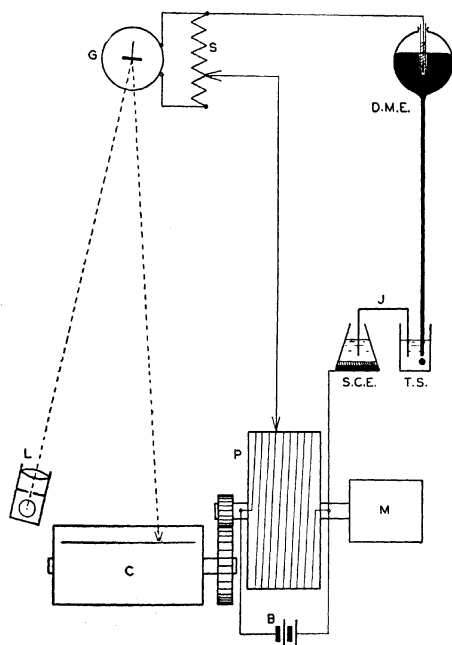


Fig. 1.—Diagram of polarograph and electrolytic cell.³

each newly forming drop exposed to the solution a fresh surface of identical properties. Under such conditions, electrolyses are independent of time and are well suited for automatic recording. Realizing this, Heyrovský and Shikata⁴ constructed a machine which increased the applied voltage at a steady rate and simultaneously recorded the corresponding current-

² J. Heyrovský, *Chem. Listy*, **16**, 256 (1922); *Phil. Mag.*, **45**, 303 (1923).

³ O. H. Müller, *Ind. Eng. Chem., Anal. Ed.*, **14**, 99 (1942).

⁴ J. Heyrovský and M. Shikata, *Rec. trav. chim.*, **44**, 496 (1925).

voltage curve. While this instrument is valuable as a timesaver, it is obviously not essential to polarographic work. Since the curves obtained by the instrument are a graphical representation of the polarization of the electrode, the machine was called a *polarograph* and the records obtained with it, *polarograms*.

Figure 1 shows a diagram of the polarograph and the electrolytic cell. The voltage from a battery, *B*, is placed across a wire of uniform resistance which is wound on the potentiometric wheel, *P*. When this wheel is rotated by the motor, *M*, a sliding contact moves along the resistance wire and increases the voltage applied to the electrolytic cell. Simultaneously, photographic paper is rotated past a slit in the camera, *C*, which records a beam of light from *L* deflected by the mirror galvanometer, *G*. By means of a shunt, *S*, the sensitivity of the galvanometer can be regulated to keep its deflections within the limits of the camera. The dropping mercury electrode, *D.M.E.*, drops in the test solution, *T.S.*, which is connected to a saturated calomel half-cell, *S.C.E.*, by means of an agar bridge or other liquid junction, *J*.

The polarograph made a large number of experimental data more easily available for the development of theories and practical applications. Eventually, sufficient knowledge was gained to extend these polarization studies to other small electrodes where the surface was mechanically renewed or where a steady state during electrolysis could be produced by some other means. All such investigations are included under the name of *polarography*.

Among the earliest studies with the dropping mercury cathode are reductions of organic substances in aqueous and organic media. Nevertheless, the major developments in polarography were made in the inorganic field, because the organic reactions were too complex and could not be directly related to available electrochemical data. It was not until the theory of the half-wave potential had been developed that organic polarography played a major role by furnishing the first experimental proof of this theory. It was found that potentials observed under certain conditions during the reduction or oxidation of reversible organic oxidation-reduction systems were no longer arbitrary but had thermodynamical significance; the half-wave potentials were identical with the potentiometrically determined oxidation-reduction potentials of these systems. This meant that the rate of all processes involved in the reaction at the surface of the dropping mercury electrode must have been fast enough to establish equilibrium conditions. While this phenomenon may be exceptional and true only in a select group of organic compounds, its occurrence establishes the relationship between polarography and other electrochemical techniques. Moreover, the existence of thermodynamically

significant potentials on the polarograms made possible a development of theories which might explain some of the more complicated reactions of organic compounds.

Unfortunately, it is practically impossible to correlate the work done by different investigators in organic polarography, not only because of a lack of uniformity in the reported results, but also because often certain necessary requirements have been neglected. Review articles and monographs have, therefore, treated mainly the inorganic applications of polarography. Müller⁵ has listed all the organic compounds investigated up to June, 1938; and Kolthoff and Lingane⁶ have included much of the organic material available up to 1940 in a monograph covering all of polarography. Despite their shortcomings, such data are valuable as preliminary information for anyone undertaking research in this field. For details concerning specific organic compounds, the original literature must be consulted. Several bibliographies,⁷ complete with titles of papers in English, German, or French are available.

The most obvious application of polarography in organic chemistry is to analytical problems because the method is both qualitative and quantitative. Since it is particularly well suited for the study of irreversible electrode reactions, the number of compounds subject to such analysis is potentially very great. In addition, polarography has been successfully applied to numerous studies of theoretical interest. The reduction of metallo-organic complexes at the dropping mercury electrode will not be discussed in this article because the available information belongs to the field of inorganic polarography.

II. THEORETICAL PRINCIPLES

1. General Introduction and Terminology

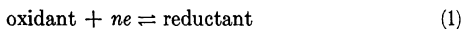
As stated above, polarography combines electrolysis with measurements of electromotive force. The electrolysis is always of short duration and never carried to completion; in fact, every effort is made to prevent a measurable alteration of the solution by the electric current. This is accomplished by the use of a microelectrode immersed in a relatively large volume of solution. The other electrode is kept large and unpolar-

⁵ O. H. Müller, *Chem. Revs.*, **24**, 95 (1939).

⁶ I. M. Kolthoff and J. J. Lingane, *Polarography*. Interscience, New York, 1941.

⁷ J. Heyrovský and J. Klumpar, *Collection Czechoslovak Chem. Commun.*, **10**, 153 (1938); J. Heyrovský, *ibid.*, **11**, 98, 667 (1939); *ibid.*, **12**, 156 (1947); J. Heyrovský and O. H. Müller, *ibid.*, **12**, 677 (1947); *ibid.*, **13**, 481 (1948). See also the industrial pamphlets: "Polarography," *E. H. Sargent and Co.*, Chicago, Ill.; "Bibliography of the Polarized Dropping Mercury Electrode," *Leeds and Northrup Co.*, Philadelphia, Pa.

izable so that any changes in current and electromotive force can be correlated directly with the processes at the microelectrode. Polarography is concerned with electrode reactions, that is, reactions involving a transfer of electrons between the electrode and components of the solution. These components are called *oxidants*, when they can accept, or *reductants*, when they can lose electrons. The electrode is *cathode* when a reduction occurs and *anode* when an oxidation takes place on its surface. During the reduction of an oxidant at the cathode, electrons leave the electrode and enter the solution with the formation of an equivalent amount of reductant. Conversely, during the oxidation of a reductant at the anode, electrons pass from the solution to the electrode and form an equivalent amount of oxidant. Since free electrons cannot exist in solution, any process of reduction at the cathode requires a simultaneous oxidation at the anode. The various types of reactions may be summarized by one single equation:



The reductant obviously differs from the oxidant merely by n electrons (e); together they form an *oxidation-reduction system*. Anions and cations, as well as uncharged molecules, can be reduced at the cathode or oxidized at the anode. The reaction written is perfectly reversible, and the starting and end products are stable in solution. Cases in which this is not true will be discussed in a later section of this article.

In organic polarography, all components of an oxidation-reduction system are dissolved in the solution. The electrode is indifferent and participates in the reactions only to the extent of furnishing or removing electrons. Since it acquires a charge indicative of the potential of the surrounding solution, it is called an *indicator electrode*. This is in contrast to inorganic polarography, where often the electrode metal itself is oxidized or metallic ions are reduced to the metal and deposited on the electrode surface. Müller⁸ has called such an electrode an *active electrode*; its potential is a function of the concentration of only one ionic species in solution. Sometimes in polarography, an electrode may be simultaneously an active and an indicator electrode, in which case great care must be exercised to segregate the potential determining factors. To characterize potentials so observed, Kolthoff and Miller⁹ have called them *mixed potentials*.

Only an extremely small number of electrons must be added or taken away in order to charge an indicator electrode to the potential of the surrounding solution. Hence reaction (1) cannot proceed unless the electrode is part of an electrical circuit by which electrons are led to or from the

⁸ O. H. Müller, *J. Chem. Education*, **18**, 65 (1941).

⁹ I. M. Kolthoff and C. S. Miller, *J. Am. Chem. Soc.*, **62**, 2171 (1940).

electrode. The equilibrium expressed in equation (1) can be formulated in accordance with the law of mass action:

$$a_{\text{ox.}} a_e^n = K a_{\text{red.}}$$

where $a_{\text{ox.}}$, a_e , and $a_{\text{red.}}$ indicate the activities of the oxidant, the electrons, and the reductant, respectively, and K is the equilibrium constant. Most studies in polarography are made with dilute solutions and the potential measurements are seldom more accurate than ± 1 mv.; hence, concentrations may be substituted for activities to obtain the simpler relationships:

$$[\text{ox.}][e]^n = K[\text{red.}] \quad \text{or} \quad \frac{[\text{ox.}]}{[\text{red.}]}[e]^n = K \quad (2)$$

in which the brackets indicate concentrations. The quantity $[e]$ may be considered an effective "concentration of electrons," an "electron fugacity," or an "electron pressure" at the surface of the electrode.

The change in free energy of a system, $-\Delta F$, with respect to the equilibrium constant, K , and the potential, E , of an electrode immersed in the system is given by the following equations:

$$-\Delta F = RT \ln K = nFE$$

where R is the gas constant, T the absolute temperature, n the change in valence, and F the Faraday. Hence:

$$E = \frac{RT}{nF} \ln K \quad (3)$$

and after substitution from equation (2):

$$E = \frac{RT}{F} \ln [e] + \frac{RT}{nF} \ln \frac{[\text{ox.}]}{[\text{red.}]} \quad (4)$$

Usually noble metals are employed as indicator electrodes, but any metal is suitable as long as its ions exist in solution in such small concentration that they do not influence the ratio of the oxidant and reductant present.

Equation (4) shows that the potential of any pure oxidant must be $+\infty$ and that of any pure reductant $-\infty$. Of course, such potentials are never obtained experimentally because solutions of the required purity cannot be obtained. Even if they existed, we could not expect such extreme potential values because of the equilibrium expressed in equation (1). An absolutely pure reductant would have to lose some electrons to charge up the electrode and thus form enough oxidant to give the potential a finite value. In practice, it has been found that a minimum concentration of about $10^{-6} M$ of both the oxidant and the reductant of a system is

necessary to make the observed potential significant. The power of an oxidation-reduction system to stabilize the electrode potential has been called its *poising action*¹⁰ by Clark.¹¹ Obviously it increases with increasing concentration of the system; at any given concentration it is greatest when the system contains equal parts of oxidant and reductant, because then changes in the concentration of each will have the smallest effect on the ratio.

If the concentrations of oxidant and reductant are equal, the last term in equation (4) becomes zero and we obtain a specific potential, the standard or *normal potential*, E_0 , of the oxidation-reduction system, expressed in terms of the electron pressure, $[e]$, as follows:

$$E_0 = \frac{RT}{F} \ln [e] \quad (5)$$

This equation shows the relationship between electrode potential and the "electron pressure" of a system. Both E_0 and $[e]$ are constants in this case, each *characteristic* of the oxidation-reduction system and, therefore, suitable for comparing different systems and for arranging them in an electromotive series.

A direct determination of either constant is not possible; only differences in the potential of two systems or electrodes can be measured. As reference electrode with a potential of zero at all temperatures, the *normal hydrogen electrode* was chosen, as explained in Chapter XXVII, p. 1714. Potentials of other systems referred to it are designated E_h . In practice, it is more convenient to measure potentials against the saturated calomel electrode; the potentials are then designated $E_{s.c.e.}$ and can be converted to E_h where required.

Let us now consider the effect of an electric current on electrode potentials that are well poised by reversible systems. If the *current density* (current per unit area of electrode surface) is small and the current of short duration, the potentials remain unchanged because the products of the electrode reaction do not materially alter the composition of either the electrode-solution *interface* (the very thin layer of solution in direct contact with the electrode) or the body of the solution with which it is in equilibrium. Establishment of this equilibrium is possible as long as the current density is small enough, even when current flows for a long time. Under such conditions, the electrode potentials change because they indicate the composition of the solution which is gradually altered by the electric current. In contrast to this, another type of potential change is

¹⁰ Poising action is analogous to the buffer action in acid-base systems, which is greatest when $\text{pH} = \text{pK}_a$. D. D. Van Slyke, *J. Biol. Chem.*, **52**, 525 (1922).

¹¹ W. M. Clark, *U. S. Public Health Repts.*, **38**, 443 (1923).

observed whenever the current density becomes so large that the interface and the body of the solution are no longer in equilibrium. Here, the rate at which equilibrium can be established is not fast enough to compensate for the alterations in the interface caused by the current. The electrodes are called polarized, because their potentials are no longer representative of the body of the solution. In general, an electrode is polarized whenever it acquires a potential different from that which it has in the absence of electrical connections. Agents which tend to prevent this polarization are called *depolarizers*. Polarography is a study of the action of depolarizers on the potential of a microelectrode during an electrolysis.

In order to ascertain which one of the electrodes in a cell is polarized, it is best to determine the potential of each electrode potentiometrically against a third electrode of known and constant potential. A second technique, used exclusively in polarography, is the use of a cell in which one electrode is of known potential, well poised, and with a large surface, while the other electrode is the dropping mercury electrode. If the current is kept small and of short duration, the large electrode has a constant potential and can serve as reference electrode. Such an arrangement has the advantages that the composition of the solution remains essentially unaltered and that all variations of current with changes in the applied voltage, apparent in a current-voltage curve, can be directly correlated with processes going on at the microelectrode.

Knowing the potential of the nonpolarizable reference electrode, E_r , we can calculate the potential of the polarized microelectrode, E_m , from the applied voltage, V , and the observed current, I , by means of Ohm's law, as follows:

$$E_m = E_r + V + IR \quad (6)$$

where R is the resistance of the electric circuit. In polarography, it is customary to consider the current positive when the microelectrode is cathode and negative when it is anode. (If the opposite terminology is used, a minus sign must be placed before IR in equation 6.)

The correction for IR becomes necessary whenever the product of the current and the resistance exceeds 1 mv. (at the usual resistance of 3000 ohms, this means where currents are greater than $0.3 \mu\text{a.}$). Hence most polarographic *current-voltage* curves must be corrected for IR before they represent *current-potential* curves. (For an example and for definition of these terms, see Müller.¹²) This correction may become very large in the presence of organic solvents which increase the resistance of the electrolyte. Fortunately, it is seldom necessary to recalculate every point on a

¹² O. H. Müller, *J. Chem. Education*, 18, 227 (1941).

current-voltage curve; usually only those points on the curve are corrected for IR which are of special interest.

The standard method of drawing current-voltage curves is illustrated by figure 2. Here, current is plotted on the vertical and the applied voltage on the horizontal axis. The line marked "galvanometer zero" shows the position of the galvanometer at rest, *i. e.*, when no current is flowing. Current above this line is positive and indicates a reduction at the microelectrode which is cathode. Negative current below this line represents an oxidation at the microelectrode which is then anode. The abscissa corresponding to zero applied voltage is of special importance because it represents the known potential of the nonpolarizable electrode to which the

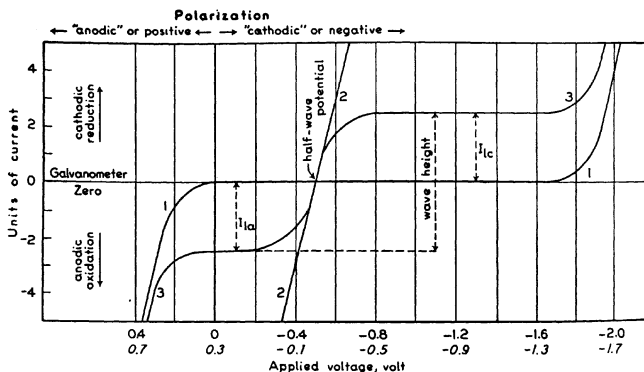


Fig. 2.—Types of current-voltage curves and standard coordinate system used in polarography.

polarization of the microelectrode is referred. To the right of this abscissa, the microelectrode is polarized to more negative potentials, and to the left of it to more positive potentials than the reference electrode. These polarizations have, unfortunately, in the past been called *cathodic* and *anodic polarizations*, respectively, a terminology which has led to some confusion because it implies a function of the electrode which may be incorrect. For instance, a cathodically polarized electrode can still function as anode as is illustrated by curve 3 of figure 2. For our purpose it is important to remember that the electron pressure of the microelectrode is gradually increased from left to right on the polarogram, and that at one point along this curve it is equal to the electron pressure of the reference electrode.

If a different reference electrode is used, identical current-voltage curves will result with a shift of the abscissa. For example, if the E_0 of the first

used reference electrode were $+0.300$ v., and the experiment had been repeated with a normal hydrogen electrode as reference electrode, the voltage axis would have become as indicated in italics in figure 2. In either case, equation (6) will give identical values for the potential of the microelectrode at any point on the curve. This example illustrates clearly that a statement of applied voltage is meaningless unless it is accompanied by information about the potential of the reference electrode which is used.

Figure 2 shows three very simple current-voltage curves under ideal conditions, which illustrate the principles of polarographic analysis. Curve 1 represents a pure *indifferent electrolyte*, so called because it serves largely to conduct current and preserve electroneutrality in solution while other substances, contained therein, react at the electrode. As may be seen, no measurable current flows through such a solution over a wide range of applied voltage, proving the absence of any depolarizer which could act in that region. At the two extreme ends of the curve, however, even this solution becomes electroactive and is decomposed as indicated by the large currents which do not reach any limits.

If a reversible oxidation-reduction system with equal proportions of oxidant and reductant is added in sufficient amount to this solution, we obtain curve 2. In this unusual case, even the microelectrode does not become polarized at the current strengths shown. A straight line is obtained with a slope determined by the resistance of the circuit. This line passes through "galvanometer zero" at a potential characteristic of the oxidation-reduction system; we have at that point a typical example of a potentiometric potential determination.¹³ This same potential is obtained from every point along this linear current-voltage curve when it is corrected for IR according to equation (6), indicating that the electrode potential remained constant in spite of the flow of current. However, this ceases to be the case if the concentration and thus the poisoning action of the oxidation-reduction system is greatly reduced, and curve 3 is obtained during the electrolysis.

As could be expected, curve 3 passes through the same point on the galvanometer zero line as curve 2 because the potentiometrically determined potential depends only on the ratio $[\text{ox.}]/[\text{red.}]$. However, the system is so poorly poised that even a small current alters the composition of the interface significantly and produces a polarization of the microelectrode, as indicated by the departure of curve 3 from the straight line, curve 2. This polarization increases with an increase in current and finally is complete as the current reaches a limit. Here the depolarizer is used up by the electric current as fast as it reaches the interface. From this point

¹³ O. H. Müller and J. P. Baumberger, *Trans. Electrochem. Soc.*, **71**, 169 (1937).

on, polarization of the microelectrode is a linear function of the applied voltage as in curve 1, until the "indifferent electrolyte" is decomposed. The magnitude of the observed anodic and cathodic *limiting currents*, I_a and I_c , depends on the effectiveness of the different depolarizers and on the rate with which they reach the microelectrode in a given time. Under certain conditions, the *limiting current* is directly proportional to the concentration of the reactive material in solution and thus serves for *quantitative analysis*. In curve 3, for instance, the anodic limiting current, I_a , tells us how much reductant, and the cathodic limiting current, I_c , how much oxidant is in solution, while the sum of these currents indicates the total concentration of the oxidation-reduction system.

The symmetrical S-shaped curve connecting the limiting currents is called a *wave* and the sum of the two limiting currents the *wave height*. The midpoint of the wave represents its steepest part, or the point of inflection, where the electrode potential is maximally poised because oxidant and reductant are present in equal concentration at the interface. This midpoint, or *half-wave potential*, characterizes the reacting material and serves for *qualitative analysis*.

Solutions in which both the oxidant and reductant of the same system are present in measurable amounts are rare in analytical polarography. Usually at the beginning of a measurement the substances present are either wholly oxidized or wholly reduced, and the corresponding polarographic waves are either all cathodic or all anodic. Furthermore, the curves differ in shape from that described as the electrode-reactions deviate from the ideal reversible type. A knowledge of the polarograms of ideal solutions is necessary, however, for an understanding of such variations.

2. Types of Current Obtained with a Dropping Mercury Electrode

As has been stated, the current flowing through the dropping mercury electrode may reach limiting values which can be used for quantitative analysis. This was recognized by Heyrovský in his earliest experiments, but the many factors which govern the magnitude of polarographic currents did not become clear until much work had been done. In the following sections, four different types of current—the condenser (residual) current, the adsorption current, the migration current, and the diffusion current—will be discussed in detail. In addition, two other types—the catalytic current and the kinetic current—which are controlled by the rates of reactions taking place at the electrode interface will also be mentioned. Because dissolved oxygen is reducible at the dropping mercury electrode,^{2,14} all electrolyses must be carried out in deaerated solutions, unless the currents measured are much greater than that of oxygen.

¹⁴ J. Heyrovský, *Časopis Českoslov. Léčárnictva*, **7**, 242 (1927).

A. CONDENSER (RESIDUAL) CURRENT

Whenever an indifferent electrolyte is electrolyzed with the dropping mercury electrode, a small residual current flows before the decomposition of the solution begins. This current rises almost linearly with the applied voltage with no indication of a wave, and it occurs even in a pure air-free solution, so that it cannot be due to the reduction of impurities. It must, therefore, be considered a non-Faradayic or "condenser" current, made appreciable by the continual charging of new mercury drops to the potential applied. It has been studied in detail by Ilkovič,¹⁵ who evaluated the polarization capacity of the dropping mercury electrode.

Metals, when submerged in an electrolyte, are covered with an electrical double layer of positively or negatively charged ions. The composition of this double layer and thus the charging current vary, depending upon the potential to which the metal is polarized. Mercury is unique in remaining electrically uncharged when it is dropping freely into a solution containing a capillary-inactive electrolyte (*e. g.*, potassium chloride and potassium nitrate). Under this condition, the mercury drops have a potential of -0.52 v. against the saturated calomel electrode. Polarized to more negative potentials, they become negatively charged, while they acquire a positive charge when they are polarized to potentials more positive than -0.52 v. This information was obtained from *electrocapillary curves* (first studied by Lippmann¹⁶), in which the surface tension of mercury, plotted against the applied voltage, usually gives a parabola with a peak near $E_{S.C.E.} -0.52$ v. At this electrocapillary maximum, the drops of mercury are largest; they decrease in size whenever the potential is increased or decreased. Any substance present in solution which can alter the electrocapillarity is called capillary-active and may have a marked effect on the polarograms.¹⁷ Besides ions which form complexes with mercury (*e. g.*, I^- , CN^- , S^{--}), a number of amines, alcohols, and ethers belong to this group.

Figure 3 illustrates the phenomenon of the condenser current. A solution of 0.1 N potassium chloride was polarographed after the oxygen had been removed by bubbling hydrogen through the solution. The zero current is not at the expected applied voltage (-0.52 v.) probably because of a last trace of oxygen which is very hard to remove, but the curve demonstrates the statements made above. Within the voltage range shown (from $+0.1$ to -1.0 v., *vs. S.C.E.*), the current is non-Faradayic¹⁵ and will

¹⁵ D. Ilkovič, *Collection Czechoslovak Chem. Commun.*, **8**, 170 (1936).

¹⁶ G. Lippmann, *Ann. Physik Chem.*, **149**, 547 (1873).

¹⁷ J. Heyrovský, F. Šorm, and J. Forejt, *Collection Czechoslovak Chem. Commun.*, **12**, 11 (1947).

be observed in all similar air-free solutions polarographed with the same capillary. The oscillations in current correspond to the formation and growth of each drop of mercury. Actually, the current starts from zero with each new drop, but the galvanometer is too slow to record the whole current sweep and oscillates merely around a mean value. Note that near -0.5 v. the oscillations are minimal because the drops of mercury are uncharged. While the condenser current is very small (less than $0.1 \mu\text{a.}$), it is nevertheless significant and must be subtracted from, or added to, the

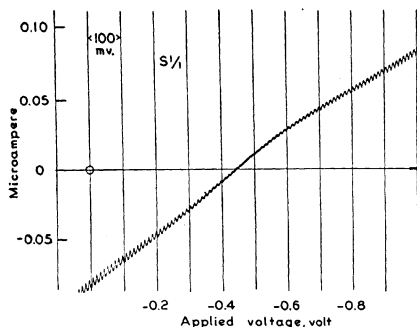


Fig. 3.—Polarogram of air-free 0.1 *N* potassium chloride solution, illustrating the condenser current.¹⁸

total current observed during the reduction of some reducible material in order that the actual current used in the reduction may be known. In very dilute solutions, neglect of this point may cause a large error.

Often the indifferent electrolyte contains traces of several impurities, so that small, almost imperceptible, waves are superimposed on the condenser current. It is customary to include these in the "residual current." As will be shown later, in practical polarographic work this residual current is automatically subtracted from the total observed current by the proper placement of tangents to the wave.

B. MAXIMA ON POLAROGRAPHIC CURVES (ADSORPTION CURRENT)

One of the peculiarities of current-voltage curves obtained with the dropping mercury electrode is that often pronounced maxima are observed which are perfectly reproducible and which can usually be eliminated by the addition of certain "maximum suppressors." A "maximum" may be

¹⁸ In this figure and in all subsequent figures, *S* indicates the fraction of the maximum galvanometer sensitivity used, the horizontal line represents "galvanometer zero," and the little "o" on the abscissa indicates zero applied voltage for the curve shown.

defined as an abnormal increase in current above the usual limiting current. It is observed most frequently at the beginning of a wave, but on occasion may develop in the middle of the straight portion of a limiting current. A maximum may be found during a reduction or oxidation of ionized or unionized, organic or inorganic substances. More than one maximum may be observed on a single current-voltage curve if the solution contains one or more electroactive substances. The maxima may be acute or rounded or may be a combination of an acute maximum followed by a

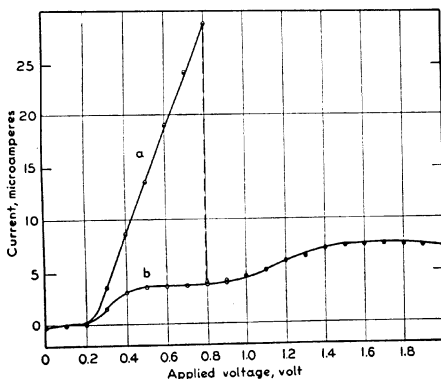


Fig. 4.—Reduction of dissolved oxygen in 0.001 *N* potassium chloride (a) in absence and (b) in presence of maximum suppressor.²⁰ The dropping mercury electrode is negatively polarized.

rounded one, depending not only on the nature and concentration of the material reduced but also on the concentration of the indifferent electrolyte and the resistance of the circuit. The heights of the maxima are variable, but it is usually possible to find optimum conditions for the production of a maximum for each electroactive substance. In general, the maxima become smaller the slower the drop time of the electrode; they usually increase with an increasing concentration of the electroactive material.

One of the most thoroughly studied maxima is that observed in the reduction of oxygen.¹⁴ In a 0.001 *N* solution of potassium chloride, this maximum is very prominent¹⁹ and has a sharp peak, as is illustrated in figure 4.²⁰ The acute maximum is of special interest because the current increases

¹⁹ E. Varasova, *Collection Czechoslovak Chem. Commun.*, 2, 8 (1930).

²⁰ O. H. Müller, *J. Chem. Education*, 18, 172 (1941).

linearly with the applied voltage until the peak is reached, when it drops discontinuously to the level of the limiting current. The slope of this straight line is equal to the reciprocal of the resistance of the cell in accordance with Ohm's law.²¹ This means that the potential of the dropping mercury electrode remains constant throughout this interval of applied voltage, and that no further polarization occurs from the start of the straight line until the peak of the maximum is reached (in Fig. 4 from about -0.3 to -0.8 v.). However, at that critical point, a further increase in the applied voltage of a few millivolts produces a sudden marked polarization of the electrode which may amount to one volt (in Fig. 4, it is 0.5 v.).

In all respects, the curve resembles current-voltage curves obtained by Müller²² with small iron electrodes which suddenly became passive (see also von Stackelberg, Antweiler, and Kieselbach²³). Müller found that an increase in external resistance produces a lowering of the maximal current and that the discontinuity (passivity) takes place at the same potential (applied voltage corrected for IR) regardless of resistance. Essentially the same effect was found in the case of polarographic maxima when the external resistance was varied.^{24, 25} In this connection should be mentioned a third similar case, observed during the oxidation of hydroquinone at the dropping mercury electrode¹² (see Fig. 9, page 1823). There, a sudden discontinuity appears in the curve with a simultaneous abrupt change in the electrode potential to a new critical value, although the wave does not show a maximum. All three instances have one feature in common: an electrode remaining depolarized or only slightly polarized during a change in the applied voltage until conditions are such that it can acquire a critical new potential to which it then will be polarized abruptly. So far, no one has been able to produce a convincing theory about the mechanism underlying these phenomena, nor is it known if the mechanism in the three cases cited is fundamentally different or not.

As a rule, maxima are an obstacle to quantitative polarographic analysis, and it is fortunate that they can be eliminated by the addition of a small quantity of a "maximum suppressor" to the solution. For instance, in figure 4, one drop of an orange G-acid fuchsin "staining solution," added to 10 ml. of electrolyte, suppressed the oxygen maximum completely. The most popular maximum suppressors have been organic indicators and dyes, but alkaloids, purine derivatives, organic acids, and gelatin, agar, and other

²¹ D. Ilkovič, *Collection Czechoslovak Chem. Commun.*, **4**, 480 (1932).

²² O. H. Müller, *Collection Czechoslovak Chem. Commun.*, **6**, 269 (1934).

²³ M. von Stackelberg, H. J. Antweiler, and L. Kieselbach, *Z. Elektrochem.*, **44**, 663 (1938).

²⁴ R. Brdička, *Collection Czechoslovak Chem. Commun.*, **8**, 419 (1936).

²⁵ J. J. Lingane, *J. Am. Chem. Soc.*, **62**, 1665 (1940).

colloids, as well as some detergents, have also been used with success. While some of these may be reduced themselves at the dropping mercury electrode, they do not interfere in the analyses as long as they are present in very small concentration. At higher concentrations, however, they render interpretation of polarograms difficult. First, the current due to the reduction of the maximum suppressor itself becomes appreciable, and second, the limiting current of the substance under investigation may actually be diminished by the suppressor for reasons unknown. Correction for the first effect is relatively simple, but the second is neither easily recognized nor corrected for unless a series of calibration curves has been prepared. Careless and excessive addition of maximum suppressors can, therefore, lead to serious error.

Adsorbable anions, acid dyes, and negative colloids which affect the ascending branch of the electrocapillary parabola are also best in suppressing "positive" maxima,²⁶ *i. e.*, maxima obtained at potentials more positive than the electrocapillary zero (the peak of the electrocapillary curve). Conversely, adsorbable cations, basic dyes, and positive colloids are best for suppressing "negative" maxima occurring in the region of the descending branch of the electrocapillary curve. If a substance is reduced at the potential corresponding to the peak of the electrocapillary curve, no maximum appears even in the absence of a suppressor. The reduction of cadmium ions is a good example.²⁷

The effectiveness of a suppressor can be expressed as the maximum dilution or minimum concentration necessary for complete suppression of a given maximum.²⁸ A better way, because the concentration of the suppressor can remain small, is to note the concentration necessary for a 50% reduction of the maximum.²⁹ More often, the reciprocal of concentration, *i. e.*, the dilution of a compound at which the maximum is half suppressed, is given in publications. This is then called the *adsorption coefficient*. Semerano³⁰ proposed a *specific suppression coefficient*, obtained by graphical means, which corresponds to the dilution of the suppressor when the maximum is reduced to $1/10$ of its maximal value.

So far, no single theory has been developed which explains all the observed data pertaining to maxima. Since most maximum suppressors are known to be surface-

²⁶ J. Heyrovský, "A polarographic study of the electrokinetic phenomena of adsorption, electroreduction and overpotential displayed at the dropping mercury cathode," *Actualités Scientifiques et Industrielles*, No. 90. Hermann, Paris, 1934.

²⁷ J. Heyrovský and E. Vascutzanu, *Collection Czechoslovak Chem. Commun.*, **3**, 418 (1931).

²⁸ N. V. Emelianova and J. Heyrovský, *Trans. Faraday Soc.*, **24**, 257 (1928).

²⁹ J. Rasch, *Collection Czechoslovak Chem. Commun.*, **1**, 560 (1929).

³⁰ G. Semerano, *Arch. sci. biol. (Italy)*, **20**, 329 (1934).

active, *i. e.*, possess the property of accumulating at interfaces, Heyrovský²⁸ concluded that "the phenomenon of maximum is one of adsorption." By adsorption, the concentration of the electroactive substance in the electrode-solution interface is increased above its value in the body of the solution, the usual polarization of the electrode is prevented, and an excess current, the *adsorption current*, can flow above the limiting current. Addition to the solution of a *more* adsorbable substance will eliminate the maximum by displacing the electroactive substance from the interface. This reasoning leads to the conclusion, which has been verified by experiment, that a good maximum suppressor should in turn show a maximum on the polarogram if it is reducible, while substances which are indifferent as maximum suppressors (*e. g.*, hydrogen peroxide or acetaldehyde) should not exhibit a maximum when reduced.

Heyrovský²⁸ considers the adsorption to be caused by the electric field around the charged drop, which is supposed to be identical with the electrokinetic potential at the interface. On the other hand, Ilkovič³¹ assumes that the charging current (condenser current) is the factor responsible for the unhomogeneous field which produces the adsorption. While the adsorption theory has not been able to explain all the factors pertaining to maxima, it has served as an excellent working hypothesis in developing the polarographic method and is still the simplest to use in discussing this complicated phenomenon.

Objections were raised to Heyrovský's adsorption theory, when it was found that a stationary mercury electrode and the surrounding solution were in active motion at relatively large current densities.^{23, 32, 33} This activity was made visible by inserting a flexible glass hair into the mercury layer and by adding minute opaque particles (sand, charcoal, etc.) to the solution.²³ The motion in the solution close to the surface of the electrode apparently was caused by local currents resulting from differences in the potential and surface tension at different parts of the mercury electrode. Antweiler³⁴ succeeded in showing that also around the dropping mercury electrode a marked streaming of solution takes place during the formation of a maximum. He concluded that the excess material necessary for the "adsorption current" is supplied to the electrode by streaming of the solution, caused by a slight difference in potential at different parts of the drop. The sudden change from the maximum to the limiting current is thought to be caused by an accumulation in the interface of indifferent ions which block further access of electroactive substances.

None of the theories proposed gives an adequate explanation of the fact that often several maxima are observed on the same side of the electrocapillary curve, nor does it consider the end products formed during the reaction. The latter are of interest especially in connection with the linear increase in current observed with many maxima (see Fig. 4, page 1799), which indicates that the electrode remained unpolarized during the formation of the maximum. Since any end products

²⁸ D. Ilkovič, *Collection Czechoslovak Chem. Commun.*, **8**, 13 (1936).

³¹ A. Frumkin and B. Bruns, *Acta Physicochim. U. R. S. S.*, **1**, 232 (1934).

³² W. Seidel, *Angew. Chem.*, **48**, 463 (1935).

³⁴ H. J. Antweiler, *Z. Elektrochem.*, **43**, 596 (1937); **44**, 719, 831, 888 (1938).

should at least produce a measurable polarization, they must have been washed away from the interface (into the body of the mercury or into the body of the solution) as fast as they were formed.

Maxima obtained during organic reductions and oxidations have been less thoroughly studied than those observed during inorganic reactions. Their suppression is often difficult. Some of them depend on the pH of the solution; they become more pronounced at the two ends of the pH scale.³⁵ Others disappear when a buffer is added.³⁶ Two different maxima may be observed if a single substance is reduced in steps at two different potentials.³⁵ On the other hand, an organic substance which can exist in two tautomeric forms may produce a maximum during the reduction of only one form (see Fig. 21, page 1847). It is likely that not all such maxima are identical in nature, and it is possible that, with a better understanding of maxima, the reaction mechanism of the reduction or oxidation of an organic substance at the dropping mercury electrode may be discerned.

C. MIGRATION CURRENT

If maxima are eliminated by a suppressor and the residual current is accounted for, there remain two major factors for supplying electroactive material to the electrode. One is migration of charged particles in the electric field and the other is diffusion of particles in a gradient produced by the removal of the material at the electrode. Heyrovský³⁷ showed that the migration current can be practically eliminated if there is added to the solution an "indifferent" electrolyte in a concentration so large that its ions carry essentially all the current. In practice, this means that the concentration of the added electrolyte must be at least 25 times as large as the concentration of the reacting material to produce a pure diffusion current. In the absence of added electrolyte, the migration current of a cation is *exalted* if it is preceded by another reduction current on the polarogram.³⁷

The mathematical treatment of these conditions has been in fair agreement with experimental data.³⁸⁻⁴⁰ It can be omitted here, since applications in organic polarography are practically nonexistent because of the need for a good conductor or adequate buffering of most reactions. The conclusions reached have been of real value because they have brought out the significance of indifferent electrolytes.

³⁵ A. Winkel and H. Siebert, *Z. Elektrochem.*, **44**, 402 (1938).

³⁶ O. H. Müller, *Cold Spring Harbor Symposia Quant. Biol.*, **7**, 59 (1939).

³⁷ J. Heyrovský, *Archiv Hem. Farm.*, **8**, 11 (1934).

³⁸ I. Šlendyk, *Collection Czechoslovak Chem. Commun.*, **3**, 385 (1931).

³⁹ J. Heyrovský and M. Bureš, *Collection Czechoslovak Chem. Commun.*, **8**, 446 (1936).

⁴⁰ J. J. Lingane and I. M. Kolthoff, *J. Am. Chem. Soc.*, **61**, 1045 (1939).

An indifferent salt is one which conducts the current but does not react with the substance under investigation, nor at the electrode within the potential range studied. Various indifferent electrolytes have been suggested which are used for many analytical procedures. Their usefulness increases with the potential range over which they are suitable. When they contain the necessary maximum suppressors and buffer mixtures for maintaining a constant *pH*, they are called *Grundlösungen*⁴¹ or *supporting electrolytes*.⁶ The buffer mixtures themselves, if concentrated enough, will conduct the current well and thus act as supporting electrolytes and eliminate the migration current.

D. DIFFUSION CURRENT

The establishment of a limiting current signifies that the reactive substance is used up by the electric current as fast as it reaches the electrode surface. If stirring effects are minimized by means of drop times in excess of 1 sec. and if there is a pure diffusion current, the electroactive material reaches the electrode entirely by diffusion in a concentration gradient caused by the removal of the material at the electrode. This gradient extends through a diffusion layer from practically zero concentration at the electrode surface to the concentration existing in the body of the solution. In the case of the dropping mercury electrode, the diffusion layer is a spherical shell, the thickness of which determines the concentration gradient at any given concentration in the solution. As the current removes more and more material, the diffusion layer should become thicker, and thus the concentration gradient should decrease with time, were it not for the fact that the diffusion shell expands in size and becomes thinner as the mercury drop grows. The observed current is therefore a complex function of the growth of the mercury drops and of the diffusion coefficient of the reactive material. Ilkovič⁴² succeeded in defining these relationships mathematically and arrived at the following equation for the diffusion current, which he verified experimentally:

$$I_d = 0.732 n F D^{1/2} C m^{3/4} t^{1/4} \quad (7)$$

where I_d is the diffusion current (in amperes) at any given time, t (in seconds), during the life of the drop. (If t is the drop time of the electrode, then I_d is the maximal diffusion current obtained during the growth of the drop.) The number of electrons involved in the reaction of one molecule of the electroactive substance is given by n , while F is the Faraday (96,500 coulombs), D is the diffusion coefficient of the electroactive material (in $\text{cm}^2 \text{ sec}^{-1}$), C its concentration (in moles per ml.), and m the weight (in g.) of mercury flowing from the capillary per second.

⁴¹ H. Hohn, *Chemische Analysen mit dem Polarographen*. Springer, Berlin, 1937.

⁴² D. Ilkovič, *Collection Czechoslovak Chem. Commun.*, **6**, 498 (1934).

It is more convenient to express I_d in terms of microamperes ($1 \mu a. = 10^{-6}$ amp.), m in the units mg. sec.^{-1} , and C as millimoles per liter.⁶ Also, the value of the Faraday is best included in the numerical constant. We then obtain:

$$I_d = 706 n D^{1/2} C m^{3/2} t^{1/6} \text{ microamperes} \quad (8)$$

Equation (8) indicates that the current increases with the sixth root of time during the life of a drop. The heavily drawn curves of figure 5 illustrate schematically such current-time curves of individual drops at different drop times. Depending on the speed of the galvanometer and the dropping rate of the electrode, curves may be recorded which vary within two extremes, from these theoretical saw-tooth curves to a practically straight horizontal line representing a constant mean current. For instance, with a torsion string galvanometer of very short period (0.01 sec.), it has been possible to record the complete current-time curves of individual drops with a drop time greater than one second. On the other hand, if the galvanometer has a very long period (more than 10 sec.) and the drop time is very short, the oscillations practically disappear. The resulting *constant* current represents a mean current which involves the same quantity of electricity as the oscillating current. In figure 5, this is shown by the horizontal line. Mathematically, this mean or average current is defined as follows:

$$\text{Mean } I_d = 1/t \int_0^t (706 n D^{1/2} C m^{3/2}) t^{1/6} dt$$

where t is the drop time. After integration, this becomes:

$$\text{Mean } I_d = 6/7 (706 n D^{1/2} C m^{3/2}) t^{1/6}$$

or:

$$\text{Mean } I_d = 605 n D^{1/2} C m^{3/2} t^{1/6} \quad (9)$$

We see, therefore, that the *mean current* is equal to $6/7$ of the *maximal* current obtained just before the drop falls off.

It is well to keep the two extreme conditions in mind when considering the curves obtained in practical polarography. Here, it is customary to work with a fairly slow D'Arsonval galvanometer (with a period of 10 sec. or over), although faster galvanometers with periods as short as one second are also used. The current consequently oscillates more or less around the mean current, as is shown by the interrupted lines in figure 5. On the recorded polarograms, any part of the oscillations can be measured with equal accuracy; but, when the operator observes the galvanometer deflections directly, only the maximum deflections can be measured accurately. For practical analyses, it makes little difference which part of the oscillations is measured as long as the investigator is consistent and works with the same setting of the capillary and with the same galvanometer. However, if the factors of the Ilkovič equation are to be evaluated or results with different capillaries are to be compared, every attempt must be made to obtain current values as near as possible to the mean current, unless an oscillographic arrangement guarantees that the maximum deflection actually measures the maximal current. As long

as the oscillations are very small, the minimum and maximum deflections as well as their average are very close to the true mean current, and any of them can be used for measurement without involving appreciable errors. But when the oscillations increase in size, the errors of all three measurements also increase. Since we know that the minimal deflection will eventually, with a fast enough galvanometer,

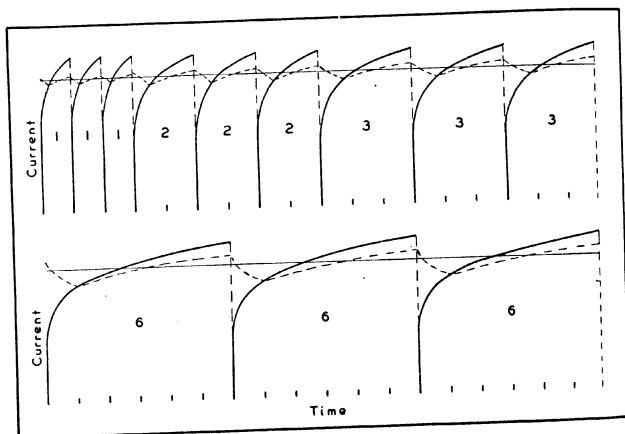


Fig. 5.—Theoretical current-time curves of individual drops of mercury at drop times of 1, 2, 3, and 6 seconds (see text).

indicate zero current, while the maximum deflection will reach $7/8$ of the mean current, we must conclude that the average deflection diverges faster from the mean current than the maximum deflection. The true mean current could then be obtained only if one measured the current corresponding to $6/7$ of the extent of the oscillations. This condition is apparently not approached with the slow galvanometers which are usually employed in polarography. For instance, Müller's results on α -oxyphenazine (Fig. 13 and Table IV), which are discussed on page 1831, indicate that the measurement of the *average* galvanometer deflection is best. Available data⁴³ for the diffusion currents of lead, measured with galvanometers of different half-periods, confirm this. One can, therefore, conclude that, with the usual polarographic equipment, the average of the maximum and minimum excursions of the galvanometer closely approaches the mean diffusion current.

The mathematical derivation of the Ilkovič equation⁴² cannot be discussed in this article. The correctness of the final equation was verified by MacGillavry and Rideal.⁴⁴ The reader is referred to Kolthoff and Lingane⁴ for a detailed discussion of diffusion to all kinds of electrodes.

⁴³ J. J. Lingane and B. A. Loveridge, *J. Am. Chem. Soc.*, **66**, 1425 (1944).

⁴⁴ D. MacGillavry and E. K. Rideal, *Rec. trav. chim.*, **56**, 1013 (1937).

The Ilkovič equation has been verified experimentally by numerous investigators and today failure of a wave to behave as required by this equation is evidence that the current is not diffusion controlled.⁴⁵ The significance of each factor of equation (9) will be demonstrated in the following paragraphs:

$I_d = Kn$.—In organic chemistry, n , the number of electrons involved in a reduction or oxidation, is usually two. In most instances these electrons appear to be transferred simultaneously, but numerous oxidation-reduction systems are now known in which they are added in two equal steps with the formation of an intermediate radical called "semiquinone" (see page 1828). In irreversible reductions, as many as six or eight electrons may be involved in the complete reduction of the molecule, and two or more steps are observed on the polarogram. For an example, see figure 19, page 1846. The wave heights of the different steps are in simple ratios to each other, and if n for one of the steps is known, the other n values can be readily calculated. To determine n for any individual wave, one may compare its height with that of a compound with a similar diffusion coefficient, polarographed under identical conditions, whose n is known. Other methods of determining n are by means of a detailed analysis of the current-potential relationships during the formation of the wave (see page 1824), or by coulometric analysis (see page 1846).

$I_d = K \cdot 605 \cdot D^{1/2}$.—In inorganic polarography, it has been possible to check the validity of the numerical constant and of the diffusion coefficient factor by comparing the value obtained by means of the Ilkovič equation with that calculated from mobilities at infinite dilution.^{6,42} So far, no critical tests have been performed in the case of organic substances. However, an apparent discrepancy observed in unbuffered solutions of quinhydrone could be explained by a consideration of diffusion coefficients, calculated by means of the Ilkovič equation (see page 1836). Hence, it seems likely that this part of the Ilkovič equation is also applicable to organic reactions.^{45a} It should be pointed out that the diffusion coefficients of reducible and oxidizable organic acids and bases may be influenced by the pH of the solution, because the ions may diffuse at a rate different from that of the corresponding undissociated molecules.

$I_d = KC$.—This is the most important relationship because it states that, all other things being constant, the diffusion current is directly pro-

⁴⁵ K. Wiesner, *Coll. Czech. Chem. Comm.*, **12**, 64 (1947); R. Bieber and G. Trümpler, *Helv. Chim. Acta*, **30**, 1109 (1947). The observation of J. K. Taylor, R. E. Smith, and I. L. Cooter, *J. Res. Natl. Bur. Stand.*, **42**, 387 (1949), that the Ilkovič equation does not strictly represent the instantaneous current at the dropping mercury electrode, does not alter this statement.

^{45a} A more recent application is the determination of the molecular weight of serum albumin by its effect on the diffusion current of methyl orange; W. Stricks and I. M. Kolthoff, *J. Am. Chem. Soc.*, **71**, 1519 (1949).

portional to the concentration of the reacting material. In principle, of course, this fact has been known since the earliest days of polarography and has been used for quantitative analysis.

The straight line relationship between diffusion current and concentration holds for organic ions and molecules, but its establishment is often complicated by the lability of the compounds, the possibility of polymerization or tautomerism, and the effect of the organic substances on the drop time when they are capillary active.

$I_d = K m^{2/3} t^{1/3}$.—This equation expresses the diffusion current as a function of two important factors, the characteristics of the capillary and the surface tension of mercury. It has been carefully tested by several investigators,^{20, 42, 46} and was found to hold for widely differing capillaries and drop times.⁴⁷ In these determinations, at a given applied voltage, the diffusion current is noted, a suitable number of drops of mercury (10 to 30, depending on size) are collected beneath the solution and weighed after being dried, and the time necessary for their formation is measured accurately with a stop watch. The drop time, t , and the weight of mercury, m , flowing through the capillary per second, can then be calculated and related to the diffusion current.⁴⁸ Obviously, these quantities are dependent on the pressure, P , of the mercury column which forces the mercury through the capillary. This is illustrated by the data of Müller,²⁰ obtained during the reduction of cadmium ions, which are given in table I. (The same relationships hold for organic compounds, as is illustrated by the results obtained with α -oxyphenazine shown in figure 13 and table IV, p. 1833. Note that the diffusion current, I_d , and the quantity, m , increase, and that drop time t decreases with an increase in pressure P , while the weight of each individual drop of mercury, W , is independent of the pressure. This means that, at constant voltage, a change in pressure will alter only the rate of dropping, not the size of the individual mercury drops.

From t and W in table I we can calculate the quantity of mercury, m , which flows through the capillary per second. This quantity is directly proportional to the pressure, P , but is independent of the applied voltage. It is now possible to calculate the quantity, $m^{2/3} t^{1/3}$, which is in a constant ratio to the corresponding I_d as shown in the seventh column, thus proving the validity of this part of the Ilkovič equation.

⁴⁶ J. Maas, *Collection Czechoslovak Chem. Commun.*, **10**, 42 (1938); J. J. Lingane and I. M. Kolthoff, *J. Am. Chem. Soc.*, **61**, 825 (1939).

⁴⁷ As shown by J. J. Lingane and B. A. Loveridge, *J. Am. Chem. Soc.*, **68**, 395 (1946). a change in the exponent of m from 0.667 to 0.64 results in an even better fit of the Ilkovič equation to results obtained with lead ion. This finding probably holds for other substances as well.

⁴⁸ For an ingenious, although somewhat complicated, device for measuring m automatically, see J. J. Lingane, *Ind. Eng. Chem., Anal. Ed.*, **16**, 331 (1944).

TABLE I^a

EFFECT OF CHANGING PRESSURE ON THE DROPPING MERCURY ELECTRODE DURING THE REDUCTION OF $5 \times 10^{-3} M$ CADMIUM SULFATE IN $0.1 N$ POTASSIUM CHLORIDE AT AN APPLIED VOLTAGE OF $-1.0 V$. (vs. S.C.E.).

P , cm.	I_d , $\times 1.24 \mu a.$	t , sec./drop	W , mg./drop	m , mg./sec.	$m^{2/3} t^{1/6}$, mg. ^{2/3} \times sec. ^{-1/2}	$\frac{I_d}{m^{2/3} t^{1/6}}$	$P/m = \kappa$
25	25.1	8.32	7.36	0.885	1.313	19.07	28.25
30	27.3	6.75	7.19	1.065	1.436	19.01	28.17
35	29.8	5.80	7.20	1.242	1.550	19.22	28.17
40	31.7	5.00	7.20	1.440	1.670	18.98	27.78
45	33.3	4.49	7.22	1.608	1.775	18.78	27.99
50	35.1	4.00	7.23	1.806	1.872	18.73	27.68
55	36.4	3.65	7.22	1.976	1.960	18.57	27.83
60	38.0	3.35	7.19	2.144	2.032	18.72	27.95
65	39.0	3.03	7.08	2.337	2.120	18.39	27.84
AVERAGE:			7.21			18.83	27.96

^a O. H. Müller, *J. Chem. Education*, **18**, 172 (1941).

As has been mentioned before, the surface tension of mercury is a function of the electrode potential and the surrounding solution. This means that drop weight W , and therefore drop time t (since m is constant at constant P) must vary with the applied voltage. Since t appears only as the sixth root in the Ilkovič equation, changing t will have a relatively small effect on the diffusion current. In fact, over the potential range from zero to about $-1.0 v$. (vs. S.C.E.), the resulting change in $m^{2/3} t^{1/6}$ and, therefore, in I_d is usually so small ($\pm 1\%$) that it can be neglected. However, at more negative potentials, the changes in $m^{2/3} t^{1/6}$ become appreciable and must be taken into account. Kolthoff and Orlemann⁴⁹ have prepared a table of values of $m^{2/3} t^{1/6}$ for different potentials, all referred to the maximum value obtained at $E_{S.C.E.} - 0.5 v$. Use of this table in organic polarography cannot be recommended, however, since many organic compounds are capillary active and thus have an influence on t . The quantity $m^{2/3} t^{1/6}$ should, therefore, always be determined at the potential at which the diffusion current is measured.

E. CURRENT CONTROLLED BY REACTION RATES

In numerous instances the current at the dropping mercury electrode is controlled largely by the rates of reactions that take place at the interface. Depending on whether such currents produce a marked maximum on the polarographic curve or just an abnormally large apparent diffusion current, one may distinguish between a catalytic current and a kinetic current.

⁴⁹ I. M. Kolthoff and E. F. Orlemann, *J. Am. Chem. Soc.*, **63**, 2085 (1941).

The former most likely involves a change in the catalytic capacity of the electrode (*e. g.*, by the formation of an amalgam), whereas the latter is produced by restoration of equilibria in the solution at the interface (with or without a catalyst) which have been disturbed by the passage of current.

Catalytic Current.—One of the least understood phenomena at the dropping mercury electrode is that of catalysis (usually of the reduction of hydrogen ions or labile hydrogen). This becomes apparent by a shift in the reduction potential of the substance reduced and by the production of maxima which cannot be suppressed in the usual way. For instance, the platinum metals,⁵⁰ calcium and magnesium,^{21, 51} and even some organic substances,⁵² lower the hydrogen overvoltage on mercury and may show one or more marked maxima on the curves.

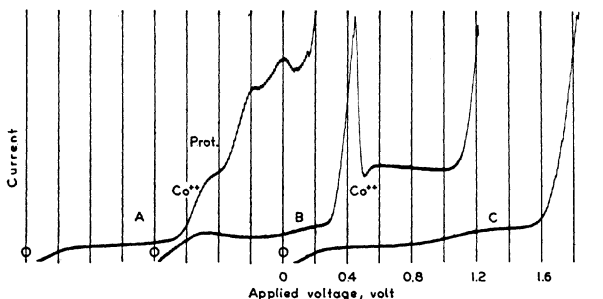


Fig. 6.—Polarogram demonstrating Brdička's reaction.

- (A) 2 ml. $8 \times 10^{-2} M$ CoCl_2 + 1 ml. 1 M NH_4Cl + 6 ml. water + 1 ml. 1 M NH_4OH + 0.1 ml. human blood plasma; (B) like A, but without plasma; (C) like A but with H_2O instead of CoCl_2 .³

From a practical standpoint the most outstanding catalytic reaction is the reduction of sulfhydryl containing compounds in the presence of cobalt (or nickel) ions in a solution 0.1 M in ammonium chloride and 0.1 M in ammonium hydroxide. This reaction, which was discovered by Brdička,⁵³ is illustrated by figure 6. Note the protein double wave (actually two

⁵⁰ I. Šlendyk, *Collection Czechoslovak Chem. Commun.*, **4**, 335 (1932). I. Šlendyk and P. Herasymenko, *Z. physik. Chem.*, **A162**, 223 (1932). P. Herasymenko and I. Šlendyk, *Collection Czechoslovak Chem. Commun.*, **5**, 479 (1933).

⁵¹ G. Kimura, *Collection Czechoslovak Chem. Commun.*, **4**, 492 (1932); O. H. Müller, *ibid.*, **7**, 321 (1935).

⁵² P. Herasymenko and I. Šlendyk, *Collection Czechoslovak Chem. Commun.*, **6**, 204 (1934). J. Pech, *ibid.*, **6**, 190 (1934). G. Cohn and I. M. Kolthoff, *J. Biol. Chem.*, **148**, 711 (1943).

⁵³ R. Brdička, *Collection Czechoslovak Chem. Commun.*, **5**, 112, 148 (1933); *Biochem. Z.*, **272**, 104 (1934).

maxima) in curve *A* which does not exist in either curve *C*, where cobalt is absent, or in curve *B*, where protein is absent. In curve *B*, the cobalt wave has a pronounced maximum which is suppressed by the protein in curve *A*. Cysteine and cystine react similarly but produce only a single wave (maximum) on the polarogram. Brdička⁵³ found that the catalyzed wave in the case of cystine is from 100 to 500 times as large as that obtained in the normal reduction of cystine in the absence of metals; he thinks of it as a catalytic reduction of hydrogen, loosened from the sulfur atom by a coordination bond between the sulfhydryl group and the cobalt. This idea is substantiated by the existence of stable complexes between cobalt and sulfhydryl-containing compounds. Furthermore, cystine and cysteine will produce a catalytic wave only in the presence of divalent cobalt, while sulfhydryl-containing compounds of higher molecular weight produce similar catalytic waves with di- or trivalent cobalt. However, this cannot be the complete explanation, since cobalt ions, whether di- or trivalent, are discharged before the catalytic wave is reached, and hence the electrode becomes a dropping cobalt amalgam electrode which by itself produces a catalytic reaction with sulfhydryl groups.⁵⁶ That both these processes may play a role is suggested by recent experiments in which the catalytic reactions of cysteine were found to be in part diffusion controlled and in part solely determined by the surface area of the electrode.^{53a}

The height of the catalytic waves is a complex function of the concentrations of cobalt and of the sulfhydryl-containing compound.⁵³ Calibration curves prepared for quantitative purposes show no straight-line relationship, but always tend toward a limiting value. Müller and Davis⁵⁴ found that these catalytic waves are proportional to the surface area of the mercury drops, which means that they are independent of the drop time of a given capillary. If the currents are expressed in microamperes per mm.² of electrode surface, results obtained with different capillaries agree very well.⁵⁴ For this evaluation it is necessary to calculate the surface area, *A*, using the formula:

$$A = 4 \sqrt[3]{\left(\frac{3W}{4\pi d}\right)^2}$$

which, at 25° C., reduces to:

$$A = 0.8517W^{2/3}$$

^{53a} O. H. Müller, *Federation Proc.*, **8**, 115 (1949).

⁵⁴ O. H. Müller and J. S. Davis, Jr., *J. Biol. Chem.*, **159**, 667 (1945); *Arch. Biochem.*, **15**, 39 (1947).

when the density of mercury, d , is 13.5340. The drop weight, W , must be determined at the applied voltage at which the catalytic current is measured.

Attempts have been unsuccessful to demonstrate catalytic activity of deposited metals on the reduction of some unsaturated organic compounds. Tachi⁵⁵ vainly sought the catalytic reduction of allyl alcohol at the dropping mercury electrode in the presence of colloidal platinum black, and the writer failed to reduce oleic acid in the presence of platinum chloride.⁵⁶

Kinetic Current.—Outside of the just-mentioned catalytic reactions, which are characterized by maxima, there has been found an ever-increasing number of reactions in which the limiting current is governed by the drop time in a manner different from that expected on the basis of the Ilkovič equation. In general, this means that the quantity of material reacting at the electrode is determined not by diffusion alone but also by some other mechanism which proceeds at a measurable rate at the interface. This extra current constitutes an apparent increase in the diffusion current and may for convenience be called a kinetic current. It is readily determined by varying the drop time of a given dropping mercury electrode and observing the deviations from the anticipated diffusion current which can be calculated by means of the Ilkovič equation. The existence of such currents has been recognized for a long time in studies of mixtures of certain reducible acids and their anions (see page 1850) and of other mobile equilibria such as keto-enol tautomerism (see page 1846). In each instance the removal of material from the interface, by electrolysis, has unbalanced an equilibrium which is restored at a measurable rate. The catalytic action of colloidal platinum and hydrogen gas on oxidant formed electrolytically at the dropping mercury electrode (see page 1823) and the enzymatic acceleration of the buffer action of the carbonate buffer (see page 1838) may serve as somewhat different examples, because here the catalysts speed up the otherwise slow restoration of the disturbed equilibrium.

The first attempt at a theoretical treatment of these phenomena was made by Wiesner,^{56a} who showed that the kinetic current was a direct function of the surface of the dropping mercury electrode (drop size) and was therefore independent of the velocity of flow of mercury from a given capillary. In further development of the theory, Brdička and Wiesner^{56b} concluded that the kinetic current must also be proportional to the rate

⁵⁵ I. Tachi, *Mem. Coll. Agr. Kyoto Imp. Univ.*, Ser. No. 17, 35 (1931).

⁵⁶ O. H. Müller, *unpublished results*.

^{56a} K. Wiesner, *Z. Elektrochem.*, **49**, 164 (1943).

^{56b} R. Brdička and K. Wiesner, *Collection Czechoslovak Chem. Commun.*, **12**, 138 (1947).

constant of the reaction and to the thickness of the surface layer (interface) in which the reaction takes place (see also page 1850). The approximate equations which they derived have been superseded by the more rigorous mathematical treatment of the kinetic current by Koutecky and Brdička,^{56c} which cannot be given here. On the basis of the equations developed by these workers, an estimation of reaction constants has become possible.

3. Capillary Used for the Dropping Mercury Electrode

For characterization of the capillary used, Müller⁵⁷ has recommended two constants which can be easily determined. One of these, the capillary constant, κ , represents the pressure (in cm. of mercury) necessary to produce a flow of mercury of one milligram per second through the capillary. The other, ρ , represents the radius of the capillary orifice. κ is easily determined as the ratio of P/m when the mercury is dropping in a solution²⁰ (last column, Table I); the small drift in the κ values can be eliminated by correction for the back pressure of mercury.⁵⁷ This correction is not necessary if, for a measured length of time, the capillary is immersed into a pool of mercury which is weighed before and after the experiment. ρ can be determined by direct measurement under the microscope or by the *critical pressure* and *drop-weight* methods developed by Müller. Since a comparison of results obtained by the last two methods is an excellent criterion for the satisfactory behavior of the dropping mercury electrode, they will be discussed in some detail.

In the first method, that critical pressure, P_c , is determined at which the flow of mercury through the capillary ceases, leaving the meniscus of the mercury just at the capillary orifice. From this critical pressure and the known surface tension of mercury, σ , the radius of the capillary at the orifice, ρ , can be calculated on the basis of the well-known equation:

$$\rho = 2\sigma/gdP_c$$

which, at 25° C., reduces to:

$$\rho = 1.508 \sigma/P_c \quad (10)$$

when ρ is expressed in microns ($1 \mu = 10^{-4}$ cm.), σ in dynes cm.⁻¹, and P_c in cm. of mercury. In table II are given ρ values obtained in this manner,⁵⁷ which are compared with the radii as measured under the microscope (accurate to $\pm 0.5 \mu$). Capillary No. 1 was factory made, while the other two were drawn in the laboratory from 0.5-mm. capillary tubing. Note that correct results were obtained when the critical pressure was measured

^{56c} J. Koutecky and R. Brdička, *Collection Czechoslovak Chem. Commun.*, **12**, 337 (1947).

⁵⁷ O. H. Müller, *J. Am. Chem. Soc.*, **66**, 1019 (1944).

TABLE II*
 DETERMINATION OF THE RADIUS, ρ , OF THE CAPILLARY ORIFICE

Methods	σ , dynes \times cm. ⁻¹	Factor	Capillaries ^b								
			No. 1			No. 2			No. 3		
			P_c , cm.	W , mg.	ρ , μ	P_c , cm.	W , mg.	ρ , μ	P_c , cm.	W , mg.	ρ , μ
Direct measurement with microscope.	27.0	14.1	8.1
Critical pressure method ^b (Eq. 10; factor/ P_c)		1.508 σ									
Capillary dropping in:											
Air	461	695	25.1 <i>25.0</i>	..	27.7 <i>27.8</i>	47.7 <i>40.5</i>	..	14.6 <i>17.2</i>	81.5 <i>74.5</i>	..	8.5 <i>9.3</i>
Water, in equilib- rium with air	374	564	21.6 <i>20.3</i>	..	26.1 <i>27.8</i>	41.7 <i>23.5</i>	..	13.5 <i>24.0</i>	74.5 <i>71.5</i>	..	7.6 <i>7.9</i>
Water, deaerated with tank nitrogen	427	644	42.0 <i>37.0</i>	..	15.3 <i>17.4</i>	73.0 <i>65.0</i>	..	8.8 <i>9.9</i>
0.1 N potassium chloride	380	573	21.1 <i>21.1</i>	..	27.2 <i>27.2</i>	40.0 <i>39.8</i>	..	14.3 <i>14.4</i>	74.0 <i>70.5</i>	..	7.7 <i>8.1</i>
Drop-weight method (Eq. 11; factor $\times W$)		1561.9/ σ									
Capillary dropping in 0.1 N potassium chloride	380	4.11	..	6.66	27.4	..	3.52	14.5	..	1.95	8.0

* O. H. Müller, *J. Am. Chem. Soc.*, **66**, 1019 (1944).

^b Values in italics were obtained by lowering the mercury reservoir, the other values by raising it.

by gradually raising the mercury reservoir and not by lowering it, and that the best agreement was found when the mercury was dropping in 0.1 N potassium chloride. The errors in water, and especially those in air, were found to be due to attachment of the mercury to the glass of the capillary.

In the drop-weight method, the capillary orifice, ρ , is determined by means of the following equation:

$$\rho = gW/2\pi\sigma$$

which, at 25° C., reduces to:

$$\rho = 1561.9 W/\sigma \quad (11)$$

when ρ is expressed in microns, W in mg., and σ in dynes cm.⁻¹. Here, too, erroneous results are obtained when the mercury becomes attached to the glass of the capillary; the measurements are, therefore, best carried out in a solution of 0.1 N potassium chloride (see Table II). As may be expected, erroneous results are obtained with the drop-weight method if the cut surface of the capillary is not perfectly horizontal, because the drops will push against the glass before they reach their full size. This may happen when the capillary tip is cut at an angle or when the capillary is not perpendicular. Such conditions become apparent when the ρ values obtained by the

drop-weight method differ by more than 0.0001 cm. from those obtained by the critical pressure method.⁵⁷ In table III data are given to illustrate this point. The diameter of the capillary used was 26.8 μ at the orifice, as determined by direct measurement under the microscope; the cut surface formed an angle of 70° instead of 90° with the longitudinal axis of the capillary. The radius at the orifice, ρ (assumed round, although it was

TABLE III^a
EFFECT OF IMPROPERLY CUT CAPILLARY ON VALUE OF ρ

Position of capillary	Critical pressure method		Drop-weight method	
	P_c , cm.	ρ , μ	W , mg.	ρ , μ
Straight	42.0	13.6	2.37	9.7
Slanted	39.5	14.5	3.32	13.7

^a O. H. Müller, *J. Am. Chem. Soc.*, **66**, 1019 (1944).

obviously oval), was determined in 0.1 *N* potassium chloride by the critical pressure and drop-weight methods, first when the capillary was vertical and the plane of the cut surface slanted, and second when the capillary was slanted to bring the orifice on a horizontal plane.⁵⁷ Note that the two methods give concordant results only in the latter case. While an improperly cut capillary may give good and reproducible results in polarographic analyses provided its position is rigidly fixed, such a capillary cannot be recommended for general use because of its extreme sensitivity to changes in position. In the past, it was difficult to determine where the fault lay, when results, hitherto reproducible, suddenly diverted from the expected. Such trouble might well have been caused by a change in the position of a capillary with a slightly slanted tip. The above preliminary check of the ρ values is, therefore, in fact a timesaver, because one can thus ascertain the proper functioning of a capillary when it is first assembled.

4. Effect of Temperature on Diffusion Current

Ilkovič has shown⁵⁸ that the effect of temperature on the diffusion current can be anticipated by a consideration of equation (9). Neglecting all temperature coefficients smaller than 0.0001 deg.⁻¹, he found that the temperature coefficient of the diffusion current is determined by the temperature coefficients of the diffusion coefficient, D , and of the viscosity of mercury, η . Kolthoff and Lingane⁶ enlarged this treatment by including the temperature coefficients of the density of mercury, d . In doing so, they overlooked, however, the fact that the drop time, t , of the Ilkovič equation (Eq. 9) is also a function of temperature, since it contains a viscosity and a

⁵⁸ D. Ilkovič, *Collection Czechoslovak Chem. Commun.*, **10**, 249 (1938).

density factor. Correcting for this error, one arrives at the following expression for the temperature coefficient of the diffusion current when the temperature is near 25° C.:

$$\frac{1}{I_d} \frac{dI_d}{dT} = 0.0034 \frac{1}{\lambda^0} \frac{d\lambda^0}{dT} \quad (12)$$

where λ^0 is the equivalent conductance at infinite dilution. Since λ^0 for most ions, excepting hydrogen and hydroxyl ions,⁵⁸ varies about 2.5% per degree change in temperature, the temperature coefficient of most diffusion currents should be near 1.5% per degree. It is to be expected that this relationship should also hold for the diffusion currents of uncharged substances. Thus it is evident that variations in the diffusion current cannot be kept below $\pm 1\%$ unless the temperature is controlled to at least $\pm 0.5^\circ$.

No accurate test of the above relationships has ever been made. In an investigation of various metal ions, Nejedlý⁵⁹ found temperature coefficients vary from 1.3 to 2.3% per degree over a wide range of temperatures. Curve *e* in figure 7 may serve as a typical example of his results. Nejedlý found that lead proved to be exceptional and showed an abnormal increase in diffusion current at higher temperatures (curve *f*, Fig. 7).

Only isolated studies of the temperature coefficient have been made in organic polarography. The results vary considerably, but it is probable that, whenever equation (12) is not satisfied reasonably well, either the current is no longer diffusion controlled or a change of state of the organic molecule is indicated. For instance, aldehydes in general show a normal temperature coefficient (about 1%) of their diffusion currents, while the temperature coefficient of the "diffusion" (?) current of formaldehyde varies, depending on pH, from 2 to 10% per degree.⁶⁰ As an example of a normal case in organic polarography, curve *d* of figure 7 has been prepared, which shows the effect of temperature on the diffusion current of pyruvic acid, reduced at pH 3.8. The temperature coefficient here corresponds to about 1.5% per degree.⁵⁶

A more complicated case is illustrated by curves *a-c* of figure 7. They represent the variation with temperature of current values during the cobalt-catalyzed reduction of protein.⁶¹ Such curves consist of a diffusion current of cobalt, followed by a protein double-wave (see page 1810). As may be seen from figure 7, the temperature coefficients of the cobalt (curve *c*) and the protein double-wave (curve *a + b*) are normal, about

⁵⁹ V. Nejedlý, *Collection Czechoslovak Chem. Commun.*, **1**, 319 (1929).

⁶⁰ E. C. Barnes and H. W. Speicher, *J. Ind. Hyg. Toxicology*, **24**, 10 (1942); F. G. Jahoda, *Collection Czechoslovak Chem. Commun.*, **7**, 415 (1935); R. Bieber and G. Trümpel, *Helv. Chim. Acta*, **30**, 706 (1947).

⁶¹ O. H. Müller, *Am. J. Physiol.*, **133**, P393 (1941).

1.7% per degree. But when components *a* and *b* of the double-wave are analyzed separately, it is found that curve *a* has a positive temperature coefficient of about 3%, while curve *b* has a negative temperature coefficient, namely -1.3% per degree.

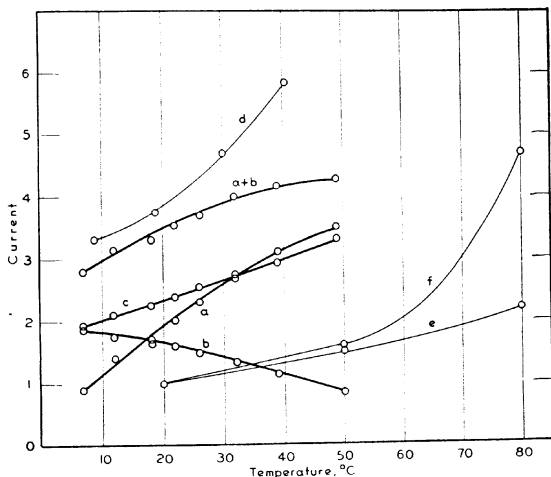


Fig. 7.—Current (in arbitrary units) plotted *vs.* temperature.

(a-c) In the catalyzed reduction of protein in a cobalt buffer: (a) first protein wave, (b) second protein wave, (a + b) protein double-wave, and (c) cobalt wave. (d) In the reduction of pyruvic acid at pH 3.8. (e) In the reduction of most metal cations. (f) In the reduction of lead ions.

5. Influence of Solvent on Diffusion Current

This problem is of special interest in organic polarography where often an organic solvent is added to the aqueous solution to dissolve enough organic material for an analysis. One can expect that the solvent may have an effect on the diffusion coefficient, D , and on the surface tension, σ . Its effect on the latter, easily recognized by a change in the drop time, may significantly alter the residual current but will have little influence on the diffusion current, because in equation (9) t is raised to the one-sixth power. On the other hand, the diffusion coefficient is generally found to be smaller in nonaqueous solvents than in water, hence the addition of nonaqueous solvents must tend to diminish the diffusion currents. This is borne out

by experiments with ethanol-water mixtures⁶² in which a minimum in the diffusion currents of different organic substances was found near a ratio of 1:1, at which ratio the viscosity of the solution also reached a maximum. As a rule, the diffusion current should be found inversely proportional to the square root of the viscosity of the solution.

6. Potential of Dropping Mercury Electrode in Reversible Oxidations and Reductions

As stated in the introduction, the potential to which the dropping mercury electrode must be polarized in order to produce an electrolysis serves for qualitative analysis. As long as the solution contains only one electroactive component, this analysis is simple; it is still possible when the solution contains several components if each is reducible at a potential sufficiently different from the other (see page 1875). However, as soon as the reduction potentials of two or more compounds come so close together that their waves on the current-voltage curves overlap, the analysis becomes difficult. Sometimes the reduction potentials can be sufficiently separated if they are altered to different degrees by changing the pH of the medium or by forming some complexes in solution, but more often a preliminary chemical separation of the components into suitable fractions is necessary before an analysis is possible. The latter is especially true in the case of organic compounds and is probably the reason why polarography has been used relatively little for practical organic analyses and why complete directions for the analysis of an organic compound have been worked out only in isolated instances.

As explained on page 1820, at any point of the S-shaped portion, or the wave of the curve, the potential of the electrode indicates the composition of the electrode-solution interface. Before the significance of the polarographic potentials was fully understood, arbitrary points on the current-voltage curves were chosen to characterize reducible substances and thus make the polarographic method suitable for qualitative analysis. For an understanding of the literature, it is necessary that these old methods be known.

At first,⁶³ that potential at which an increase of 1 mv. in the applied voltage produced an increase in the current of 10^{-8} amp. was called the "deposition potential" of the (metallic) ion. When it was found that the curves represented exponential functions, Heyrovský and his school compared the applied voltage corresponding to those points at which tangents

⁶² O. H. Müller, *Trans. Electrochem. Soc.*, **87**, 441 (1945); R. Pasternak and H. v. Halban, *Helv. Chim. Acta*, **29**, 190 (1946).

⁶³ J. Heyrovský, *Chem. Listy*, **16**, 256 (1922); *Phil. Mag.*, **45**, 303 (1923).

of equal slope made contact with the curve.⁶⁴ For convenience, they chose a 45° tangent; the corresponding potentials were called "reduction potentials." Until the "half-wave potential" was developed,⁶⁵ this was the most popular method of characterizing reactive material; it is still used occasionally when the half-wave potential method is not applicable, as in the case of maxima or when the currents are so great that the plateau of the diffusion current cannot be reached. In addition to these methods, the following modifications were proposed: (1) Semerano⁶⁶ suggested as characteristic the point of maximum curvature of the polarographic wave, which he obtained by placing a tangent at 35° 16' to the curve. (2) Shikata⁶⁷ took as the reduction potential that value of the applied voltage at which an increase of 10 mv. produced a rise in current of 1.9×10^{-8} amp.

The most outstanding shortcoming of all these methods is that the potentials change with the concentration of the reducible substance, with the sensitivity of the galvanometer, and with the drop time and the rate of flow of the mercury.⁶⁸ The exact conditions for standardization of the values always had to be stated in order to make a reproduction of the results possible. LeBlanc's method of extrapolation to zero current⁶⁹ would have been better than the above were it not for the fact that the extrapolations are very uncertain because only a very small portion of the wave resembles a straight line.

It was a great step forward when Heyrovský and Ilkovič⁶⁵ clarified the significance of the polarographic wave and suggested the *half-wave potential* for the characterization of the reacting material. This half-wave potential is independent of the concentration of the reacting material, the drop time of the electrode, and the galvanometer sensitivity. It is, furthermore, closely related to the potentials obtained in potentiometric studies, as will be illustrated in this chapter. It should be pointed out, however, that the half-wave potential is only of real value if the reaction at the dropping mercury electrode is reversible and the polarographic wave has a perfect symmetrical S shape. Often in organic reactions, this is not the case and the half-wave potential will remain as arbitrary as the former tangent potentials.

A. OXIDATIONS AND REDUCTIONS IN WELL-BUFFERED SOLUTIONS

The polarographic wave in a thermodynamically reversible reaction is a perfect equivalent of a potentiometric oxidation-reduction titration curve,

⁶⁴ J. Heyrovský, *Rec. trav. chim.*, **44**, 488 (1925).

⁶⁵ J. Heyrovský and D. Ilkovič, *Collection Czechoslovak Chem. Commun.*, **7**, 198 (1935).

⁶⁶ G. Semerano, *Gazz. chim. ital.*, **62**, 518 (1932).

⁶⁷ M. Shikata, *Mem. Coll. Agr. Kyoto Imp. Univ.*, Ser. No. 4, 1 (1927).

⁶⁸ O. H. Müller, *Chem. Revs.*, **24**, 95 (1939).

⁶⁹ A. Winkel and G. Proske, *Ber.*, **69**, 693 (1936).

as may be seen by inspection of figure 8. (For other illustrations see the paper of Müller.⁷⁰) Yet both types of curves signify different conditions.⁷¹ The potentiometric titration curve, b, indicates the alteration of a solution brought about by the addition of a suitable oxidant or reductant. The polarographic curve, a, on the other hand, indicates the change in the composition of the interface, brought about by the addition or removal of electrons in an essentially unaltered solution. We may therefore consider the polarographic wave as representing an *electron titration of the interface*,

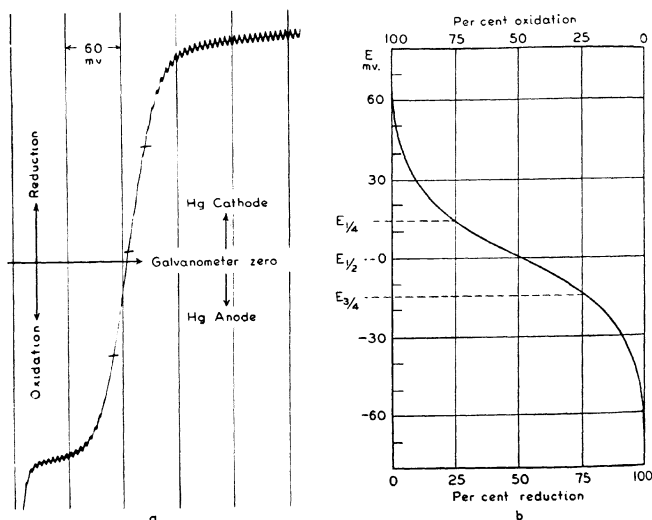


Fig. 8.—(a) Polarogram of quinhydrone in a buffered solution (see also Fig. 2, curve 3) representing a direct titration of the electrode-solution interface, while body of solution remains practically unchanged. (b) Typical potentiometric oxidative or reductive titration curve representing changes throughout body of solution.⁷¹

where the oxidizing or reducing agent is the electrode itself which removes electrons from or adds them to the electroactive material in the interface.⁷¹ Just as the potentiometric potentials are defined by the relative concentrations of oxidant and reductant in solution, so the polarographic potentials are defined by the relative concentration of oxidant and reductant in the interface. Consequently, we can apply the previously developed equation

⁷⁰ O. H. Müller, *J. Chem. Education*, **18**, 65 (1941).

⁷¹ O. H. Müller, *Cold Spring Harbor Symposia Quant. Biol.*, **7**, 59 (1939).

(4) combined with (5) to polarographic curves by substituting for the concentrations existing in the body of the solution those existing at the interface, indicated by the subscripts, i , as follows:

$$E_{D.M.E.} = E_0 + \frac{RT}{nF} \ln \frac{[\text{ox.}]_i}{[\text{red.}]_i} \quad (13)$$

If we now consider a polarographic wave in which all factors influencing the current, except diffusion, have been eliminated, the following conclusions can be drawn. As long as no current flows, the concentration of a reducible or oxidizable substance must be the same in the interface as in the body of the solution. Whenever the applied voltage is large enough to bring about an oxidation or a reduction, some of the reactants at the interface are used up and must be replenished from the body of the solution by diffusion. On the other hand, the newly formed products do not remain long at the interface but diffuse away from the electrode into the solution or into the electrode material. Further increase in the applied voltage will produce a condition in which all of the reactant reaching the interface is used up, leaving only the newly formed product. The current flowing under such circumstances is the diffusion current. At the midpoint of a wave, or the half-wave potential, the concentrations of oxidant and reductant at the interface are equal if their diffusion rates are identical. Consequently, the half-wave potential is closely related to the normal potential as established potentiometrically. On the basis of such reasoning, Heyrovský and Ilkovič⁶⁵ were able to derive the complete equation for the polarographic wave and to establish the significance of the half-wave potential.

When current is governed only by the rate of diffusion of a reactant, it is a function of the difference in concentration between the interface and the body of the solution. In the case of a reduction, the current is positive and can be expressed as follows:

$$I = k D_{\text{ox.}}^{1/2} ([\text{ox.}] - [\text{ox.}]_i) \quad (14)$$

where I is the current, $D_{\text{ox.}}$ the diffusion coefficient of the oxidant, and $[\text{ox.}]$ the concentration in the body of the solution, while $[\text{ox.}]_i$ is the concentration at the interface. The constant, k , is given by the Ilkovič equation. When the cathodic diffusion current is maximal, $[\text{ox.}]_i$ becomes negligibly small compared with $[\text{ox.}]$ and:

$$I_{dc} = k D_{\text{ox.}}^{1/2} [\text{ox.}] \quad (15)$$

Therefore:

$$[\text{ox.}]_i = (I_{dc} - I)/k D_{\text{ox.}}^{1/2} \quad (16)$$

On the other hand, the concentration of reductant at the interface is equal

to that existing in the body of the solution plus that fraction of the additional reductant formed by the current which has not diffused away. This can be expressed as follows:⁷²

$$[\text{red.}]_i = [\text{red.}] + I/kD_{\text{red.}}^{1/2} \quad (17)$$

The negative anodic diffusion current, $-I_{da}$, is reached when the concentration of reductant at the interface is zero, or:

$$-I_{da} = k D_{\text{red.}}^{1/2} [\text{red.}] \quad (18)$$

Combining (17) and (18), we obtain:

$$[\text{red.}]_i = (I - I_{da})/kD_{\text{red.}}^{1/2} \quad (19)$$

We can now substitute from equations (16) and (19) in equation (13) and obtain:

$$E = E_0 + \frac{RT}{nF} \ln \frac{(I_{dc} - I)}{(I - I_{da})} + \frac{RT}{nF} \ln \frac{D_{\text{red.}}^{1/2}}{D_{\text{ox.}}^{1/2}} \quad (20)$$

This is the most general equation for the polarographic wave involving reversible reactions. It should hold for anodic waves, where $I_{dc} = 0$ and I is negative, and for cathodic waves, where $I_{da} = 0$ and I is positive. In addition it is applicable to any single wave in which the current is partly anodic and partly cathodic, *i. e.*, where I_{da} and I_{dc} have values other than zero.

By definition, the half-wave potential corresponds to the midpoint of the wave, that is, where $I = (I_{dc} + I_{da})/2$. If we substitute this value for I in equation (20), the middle term drops out:

$$E_{1/2} = E_0 + \frac{RT}{nF} \ln \frac{D_{\text{red.}}^{1/2}}{D_{\text{ox.}}^{1/2}} \quad (21)$$

The last factor in this equation is a constant for any given case and can usually be neglected because the ratio of the diffusion coefficients of oxidant and reductant is so close to unity that any correction falls within the experimental error. Equation (21) then means that the half-wave potential, whether measured on a cathodic wave or anodic wave, or on a wave which is partly cathodic and partly anodic, should be equal to the normal electrode potential of the reacting system.

This conclusion is of great importance because it serves as a *criterion of reversibility in polarography*: A substance is polarographed once when it is present in the oxidized form and once when it is present in the reduced form. If the system is reversible, the half-wave potentials of the waves

⁷² Similar to equation (14) this equation can also be written: $-I = k D_{\text{red.}}^{1/2} ([\text{red.}] - [\text{red.}]_i)$; the current is negative because it is anodic.

obtained are identical and equal to the potentiometrically determined normal potential of the same system.⁷³

This is illustrated by the polarogram shown in figure 9, which was prepared in three stages.⁷⁴ (1) Curve *a* was obtained first with an air-free solution of quinone in a McIlvaine buffer of pH 7; note that the wave is all

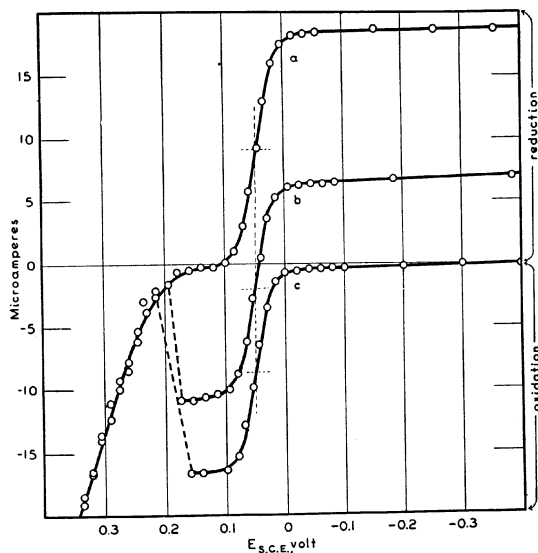


Fig. 9.—Current-potential curves obtained at pH 7.⁷⁴ (a) Quinone; (b) quinone partly reduced by platinized asbestos and hydrogen gas; (c) quinone fully reduced by platinized asbestos and hydrogen gas.

cathodic. (2) The quinone was then reduced by bubbling hydrogen through the solution in which platinized asbestos had been present throughout; after the chemical reduction had proceeded for a short period of time, curve *b* was prepared, showing that about half of the quinone had been reduced to hydroquinone;⁷⁵ here we have a continuous wave which is partly

⁷³ O. H. Müller and J. P. Baumberger, *Trans. Electrochem. Soc.*, **71**, 181 (1937).

⁷⁴ O. H. Müller, *J. Chem. Education*, **18**, 227 (1941).

⁷⁵ If for this experiment colloidal platinum is used in a solution saturated with hydrogen, abnormally large anodic waves will be produced because of the catalytic reduction of newly formed oxidant at the electrode-solution interface, which again must be oxidized electrolytically; K. Wiesner, *Z. Elektrochem.*, **49**, 164 (1943).

anodic and partly cathodic. (3) The reduction by means of hydrogen gas was then continued until all of the remaining quinone was changed into hydroquinone; at this point curve *c* was obtained which is entirely anodic. All three curves have been corrected for *IR*; the half-wave potentials, indicated on the polarogram, are identical.^{75a}

It may be noticed that currents obtained in the oxidation of hydroquinone suddenly drop to very small values when critical potentials are reached. This is prob-

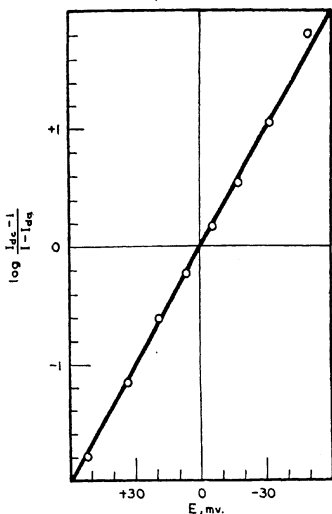


Fig. 10.—Graph of $\log (I_{dc} - I)/(I - I_{da})$ vs. the electrode potential (data from Fig. 9).⁷⁴

ably caused by a change in the dropping mercury electrode from an indicator electrode, showing the ratio of quinone to hydroquinone, to an active electrode (see page 1790), producing mercurous ions which form a complex with the phosphate ions of the buffer. The relationship of this phenomenon to the well-known maxima (page 1800) still needs to be investigated.

The wave heights of the quinone and hydroquinone curves of figure 9 are in a ratio of 15.3 to 14.1. If none of the quinone was destroyed during the reducing process and if the adsorption of oxidant and reductant on the platinized asbestos was equal, this ratio must express the ratio of the square roots of the diffusion coefficients of quinone and hydroquinone. Hence, according to equation (21) the polarographic half-wave potentials and the potentiometrically determined E'_0 of quin-

hydrone in the same buffer should differ by about 1 mv. This difference is so small that it falls within the experimental error of the measurements.

For a complete analysis of the current-potential curves, it is customary to plot the logarithmic factor of the middle term of equation (20) (converted to common logarithms) against the electrode potential, E .^{71, 76} A straight line should result, with a slope equal to $2.3 RT/nF$, from which can be found the number of electrons involved in the reaction. At 30° C.,

^{75a} In an ingenious modification of this procedure a current alternator is employed simultaneously with the polarograph; M. Kalousek, *Collection Czechoslovak Chem. Commun.*, **13**, 105 (1948).

⁷⁶ J. Tomeš, *Collection Czechoslovak Chem. Commun.*, **9**, 12 (1937).

the slope is equal to 0.0601 v., if n is unity; it is twice as steep, or 0.030 v., if $n = 2$.

The curves in figure 9 were obtained at 30° C. The corresponding graph of $\log (I_{dc} - I)/(I - I_{dc})$ vs. the potential of the dropping mercury electrode, E , shown in figure 10, gives the expected straight line with the theoretically correct slope of 0.030 v.⁷⁴ The evaluation of the continuous anodic-cathodic curve b of figure 9 is tedious because the current changes sign and two different diffusion currents have to be considered. Since it is much simpler and will give identical results for purposes of this graph, the writer has in the past treated such a polarographic wave as completely cathodic; in other words, the current I was considered equal to zero at the start or bottom of the wave and equal to I_d at the end or top of the wave. Equation (20) then takes the simpler form:

$$E = E_0 + \frac{RT}{nF} \ln \frac{I_d - I}{I} \quad (22)$$

where I and I_d are always positive. In this case, $\log (I_d - I)/I$, plotted against the potentials, E , will give the same straight line as above.

The results obtained with the quinone-hydroquinone system were duplicated with other reversible systems, leaving no doubt that the foregoing conclusions were essentially correct. They were verified also in the inorganic field, when curves obtained with dropping amalgam electrodes were studied,^{77, 78} and when conditions were finally found under which the reductions of multivalent ions proceeded reversibly at the dropping mercury electrode.⁷⁹⁻⁸¹

The identity of polarographic and potentiometric potentials in the examples mentioned demonstrates that all rates involved in the actual electrode reaction are so fast that equilibrium is established in spite of the fact that considerable current is flowing as the mercury drops grow to their full size. Thus the above examples serve as *ideal* patterns, and any deviations therefrom should be useful in achieving a full understanding of the nature of the electrode reactions. Before discussing such deviations we must consider the possible mechanisms involved in these ideal organic reductions and oxidations.

Let us take, for example, a reaction in which the addition of two electrons changes the oxidant, R , into the corresponding reductant, R^{--} . Here R^{--}

⁷⁷ J. J. Lingane, *J. Am. Chem. Soc.*, **61**, 976 (1939).

⁷⁸ J. Heyrovský and M. Kalousek, *Collection Czechoslovak Chem. Commun.*, **11**, 464 (1939).

⁷⁹ R. Strubl, *Collection Czechoslovak Chem. Commun.*, **10**, 475 (1938).

⁸⁰ M. Kalousek, *Collection Czechoslovak Chem. Commun.*, **11**, 592 (1939).

⁸¹ J. J. Lingane, *Chem. Revs.*, **29**, 1 (1941).

represents a divalent anion, or the completely ionized form of a dibasic acid. Its concentration is, therefore, subject to the hydrogen-ion concentration in the solution, which governs the degree of ionization of the acid according to the following equations:

$$K_{a_1} = \frac{[H^+][RH^-]}{[RH_2]} \text{ and } K_{a_2} = \frac{[H^+][R^{--}]}{[RH^-]} \quad (23)$$

in which K_{a_1} and K_{a_2} are the two dissociation constants of the acid. We could thus express equation (4) in terms of R , RH_2 , H^+ , K_{a_1} and K_{a_2} , but the new equation would still be impractical because the concentration of R^{--} or RH_2 cannot be determined directly in solution. A similar situation would hold if the organic substance were a base and we were trying to determine the concentration of the cation. Clark and Cohen⁸² have shown that these difficulties can be overcome if the equation is expressed in terms which represent the *sum* of all the forms in which the oxidant or reductant can appear. These summation terms we shall designate by capital letters, Ox., and Red.; in the above case this means that $RH_2 + RH^- + R^{--} = \text{Red.}$, and $R = \text{Ox.}$ Combining this with equations (23), (4) and (5), we obtain:

$$E = E_0 + \frac{RT}{2F} \ln \frac{[\text{Ox.}]}{[\text{Red.}]} + \frac{RT}{2F} \ln (K_{a_1}K_{a_2} + K_{a_1}[H^+] + [H^+]^2) \quad (24)$$

Equation (24) reduces to $E = E_0$ when $[\text{Ox.}] = [\text{Red.}]$ and $[H^+] = 1$, since all K_a values are negligibly small compared with this $[H^+]$.

Like equation (4), equation (24) can be extended to concentrations existing at the interface, by substituting $[\text{Ox.}]_i$ and $[\text{Red.}]_i$ for $[\text{Ox.}]$ and $[\text{Red.}]$. In addition, we must also substitute $[H^+]_i$ for $[H^+]$. In applying such an equation to polarographic curves, we consider as a first approximation that the diffusion coefficients of Ox. and Red. are those of a single species and that their ratio is near unity at any *pH*. We can then proceed as with equation (13) and develop the following equation in which, because it is negligible, the diffusion coefficient factor is omitted:

$$E = E_0 + \frac{RT}{2F} \ln \frac{(I_{dc} - I)}{(I - I_{da})} + \frac{RT}{2F} \ln (K_{a_1}K_{a_2} + K_{a_1}[H^+]_i + [H^+]_i^2) \quad (25)$$

If the *pH* of the interface is kept constant by a suitable buffer, the last term in this equation is also constant and can be incorporated in the E_0 term as follows:

$$E = E'_0 + \frac{RT}{2F} \ln \frac{(I_{dc} - I)}{(I - I_{da})} \quad (26)$$

⁸² W. M. Clark and B. Cohen, *U. S. Pub. Health Repts.*, **38**, 666 (1923).

At the half-wave potential, the logarithmic term is zero and we have $E_{1/2} = E'_0$. That this is so has already been demonstrated in figure 9.

Clark and Cohen,⁸² in their potentiometric studies, have shown that it is possible to determine the dissociation constants of the reductant or oxidant from a graph in which the E'_0 is plotted against pH . In potentiometry this is somewhat laborious because the concentrations of Ox. and Red. have to be made equal (see Eq. 24) to obtain $E = E'_0$. In polarography such graphs are much simpler to prepare, since $E_{1/2} = E'_0$, regardless of the relative concentrations of Ox. and Red. in the body of the solution. It must be borne in mind, however, that the potential measurements in polarography are as a rule much less accurate than in potentiometry.

The reversibility of oxidation-reduction systems has been proved repeatedly by the identity of their respective cathodic and anodic half-wave potentials, but relatively few publications have appeared in which the potentials were measured at more than two or three pH values. Since they give much needed information and are relatively simple to obtain polarographically, the preparation of pH -potential curves over as large a pH range as possible is urgently recommended in further work.

It has been assumed in the foregoing discussion that the two components of an oxidation-reduction system are both sufficiently soluble and stable to produce well-defined anodic and cathodic waves. This assumption was justified in dilute solutions of most systems studied. In more concentrated solutions, however, after chemical reduction of the oxidized form of a system, part of the reduced compound, *e. g.*, the leuco form of a dye, may precipitate out because it is less soluble than the oxidized form, or may form a nonreactive complex with the medium in which it is dissolved. The anodic wave will therefore be considerably smaller than the corresponding cathodic wave or may be completely absent so that the polarographic reversibility of a compound cannot be established with certainty. An example of this was found by Müller⁸³ with methylene blue in a phosphate buffer. If a compound is stable in the oxidized but very unstable in the reduced form, it may give an ideal cathodic wave, every point of which fits the theoretical equation for a reversible reaction, but will not give the corresponding anodic wave with the same half-wave potential. If the reduction product decomposes at a slow rate, its presence may still be revealed by a quick polarographic analysis. The presence of an anodic wave, although smaller than the cathodic wave, but with the same half-wave potential, may assure us of the reversibility of the primary electrode reaction. An example of this was found by Wiesner⁸⁴ with adrenochrome.

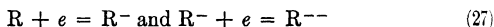
⁸² O. H. Müller, *Trans. Electrochem. Soc.*, **87**, 441 (1945).

⁸⁴ K. Wiesner, *Biochem. Z.*, **313**, 48 (1942).

B. STEPWISE OXIDATIONS AND REDUCTIONS IN WELL-BUFFERED SOLUTIONS
(SEMIQUINONE FORMATION)

In the organic reactions so far considered, the transfer of the two electrons was assumed to be simultaneous. This assumption was considered correct for all organic reactions, since the experimental data fitted the theoretical titration curves constructed on the basis that $n = 2$. However, in 1931, Friedheim and Michaelis⁸⁵ and Elema⁸⁶ found independently that pyocyanine in acid solution added the electrons in two separate steps, forming first an intermediate radical with an odd number of electrons before the final reduction product. More compounds were found which showed a similar behavior, and Michaelis⁸⁵ called these intermediates semiquinones. It is possible that all organic reductions and oxidations of reversible systems go by way of semiquinones, but in most of them the semiquinones are so unstable that their existence cannot be demonstrated.

The formation of semiquinones becomes clear by a consideration of the following reduction:



which takes place in two separate steps of one electron each. Here R is the oxidant (ox.), R^- the semiquinone (sem.), and R^{--} the reductant (red.). To avoid a consideration of the dissociation constants of the acids formed by the union of these anions with hydrogen ions, we shall again use capital letters to indicate summation terms (see page 1826) and discuss only potentials obtained at constant pH. If true equilibrium exists, the potential must then be given by a modification of equation (24), as follows:

$$E = E'_0 + \frac{RT}{2F} \ln \frac{[\text{Ox.}]}{[\text{Red.}]} \quad (28)$$

The concentrations of Red. and Ox. are modified according to the equilibrium of the dismutation process:



which may be expressed by the dismutation constant:

$$K_d = ([\text{Red.}][\text{Ox.}])/[\text{Sem.}]^2 \quad (30)$$

The reciprocal of K_d is called the semiquinone formation constant and is designated by k_s . If K_d is very large (k_s very small), the amount of semiquinone present becomes negligible and the reaction will be that of a system with no intermediate step of reduction.

⁸⁵ E. Friedheim and L. Michaelis, *J. Biol. Chem.*, **91**, 355 (1931); L. Michaelis, *ibid.*, **92**, 211 (1931).

⁸⁶ B. Elema, *Rec. trav. chim.*, **50**, 807 (1931).

Müller⁸⁷ obtained the following equation for the polarographic potential which takes into account the formation of semiquinone:

$$E = E'_0 + \frac{RT}{2F} \ln \frac{I_d - I}{I} + \frac{RT}{2F} \ln \frac{\sqrt{4K_d I_d^2 - (4K_d - I)(I_d - 2I)^2} + (I_d - 2I)}{\sqrt{4K_d I_d^2 - (4K_d - I)(I_d - 2I)^2} - (I_d - 2I)} \quad (31)$$

This equation for polarographic curves is the exact parallel of the equation of Michaelis and Schubert⁸⁸ for potentiometric titration curves and demon-

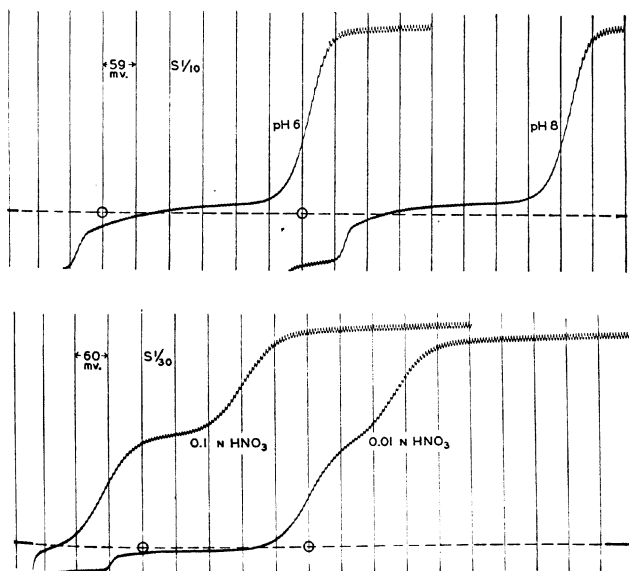


Fig. 11.—Polarograms of α -oxyphenazine in McIlvaine buffers of pH 6 and 8, and in 0.1 and 0.01 N HNO_3 solutions, showing two-step reduction in the acid solutions.⁸⁷

strates the symmetry of the curve around the midpoint of the wave. The shape of the wave varies with the value of K_d or k_t .

A good example of the effect of semiquinones on polarographic curves

⁸⁷ O. H. Müller, *Ann. N. Y. Acad. Sci.*, **40**, 91 (1940).

⁸⁸ L. Michaelis and M. P. Schubert, *Chem. Revs.*, **22**, 437 (1938).

is given by α -oxyphenazine.^{87, 89} Figure 11 clearly illustrates that a single wave is obtained at pH 6 or 8, while in acid solution two distinct waves of equal height appear which are more widely separated the stronger the acid. The half-wave potentials (or the $E_{1/4}$ and $E_{3/4}$ potentials during semi-quinone formation) obtained when the concentration of α -oxyphenazine was $5 \times 10^{-4} M$ or greater are plotted against pH in figure 12. As may be seen,

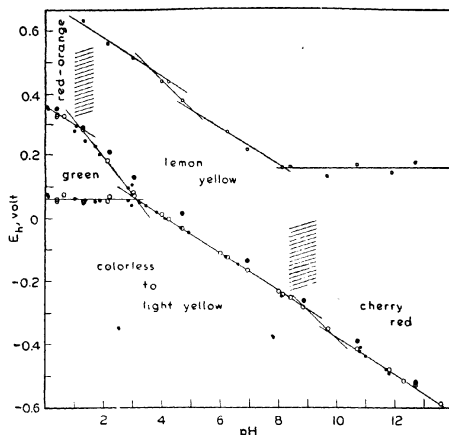


Fig. 12.—Comparison of potentiometric and polarographic data for α -oxyphenazine.

●, Potentials determined potentiometrically by Michaelis *et al.* ○, Polarographic half-wave potentials obtained with 5×10^{-4} and $10^{-3} M$ solutions. ●, Polarographic half-wave potentials obtained with $10^{-4} M$ and less concentrated solutions. ○, Polarographic half-wave potentials of small anodic wave, the significance of which is still unknown.⁸⁹

they show excellent agreement with data obtained potentiometrically. In more dilute solutions of α -oxyphenazine, however, discrepancies were found which will be discussed in some detail because they indicate an important difference between polarographic and potentiometric data.

C. ANOMALOUS WAVES OF OXIDATION-REDUCTION SYSTEMS

Under suitable conditions, many reversible organic oxidation-reduction systems⁷⁸ show an "anomalous" wave on the polarogram in addition to the one which might be expected on the basis of potentiometric data. In a

⁸⁹ O. H. Müller, *J. Biol. Chem.*, **145**, 425 (1942).

study of α -oxyphenazine, Müller⁸⁹ showed that this anomalous wave is not due to an impurity and must be ascribed to some yet unknown modification or "tautomer" of the compound studied. Brdička⁹⁰ thinks that the anomalous wave found in the case of methylene blue is produced by adsorption. However, to account for the results obtained with riboflavin, he modified this view, by assuming⁹¹ a tautomeric form of this compound which is "adsorption-inactive." Because the anomalous wave is most prominent in the thiazines which, at great dilution, also show spectroscopic anomalies and an effect of concentration on the potentiometrically determined potentials, Müller⁹² suggested a common cause. Obviously much additional work is necessary before this phenomenon is clearly understood.

Since this anomalous wave is peculiar to polarographic data, it must be caused by the dropping mercury electrode. Indeed, it was found that changing the drop time of the electrode or using different electrodes produced marked alterations in the height of this anomalous wave which could not be predicted from the Ilkovič equation.⁸⁹ This effect is shown in figure 13, the curves of which were obtained with the same 0.0001 *M* solution of α -oxyphenazine in 0.1 *N* sulfuric acid. Three different capillaries were used, one of them at two different pressures of mercury, and so covered a range of drop time from 1.53 to 7.7 seconds. The galvanometer sensitivities were adjusted so that all curves could be measured with approximately the same accuracy. It becomes obvious from an inspection of the polarograms that the slower the drop time, the smaller the discrepancy due to the anomalous wave. In spite of all this, the sum of the diffusion currents of the two waves, the first one of which includes the anomalous wave, fulfills the requirements of the Ilkovič equation (Eq. 9) fairly well, as may be seen from table IV. The italicized capital letters refer to the curves of figure 13. In the following columns are listed the number of the capillary used, its characteristic constant, κ (page 1813), the pressure of mercury, *P*, and the corresponding drop time, *t*, at an applied voltage of -0.3 v. (vs. S.C.E.). Since $P/\kappa = m$, the value of $m^{1/2} t^{1/2}$ can be calculated. The ratio between the diffusion current and this factor must be a constant if the Ilkovič equation holds.

As has been stated earlier (page 1806), for best results the true mean current should be known. In the present case, the average of the oscillations (measured as in Curve B, Fig. 13), the maximum deflection (as in Curve D, Fig. 13), and the current corresponding to $\frac{2}{7}$ of the oscillations (see page 1806) have been measured. We see from table IV that the first measurement is best in this case, since the four results agree with an ac-

⁸⁹ R. Brdička, *Z. Elektrochem.*, **48**, 278 (1942).

⁹¹ R. Brdička, *Z. Elektrochem.*, **48**, 686 (1942).

⁹² O. H. Müller, *Trans. Electrochem. Soc.*, **87**, 441 (1945).

curacy of $\pm 3\%$. Note that the difference between the three methods of measurement becomes negligible when the fast capillary, No. 2, is used.

Another phenomenon to which attention should be called is that dyes of the eosin group will suppress the polarographic waves of reversible oxidation-reduction systems. In reductions, part of the original wave is shifted to more negative potentials, whereas in oxidations only a diminution of the wave is observed when eosin is added. This suppression depends on the

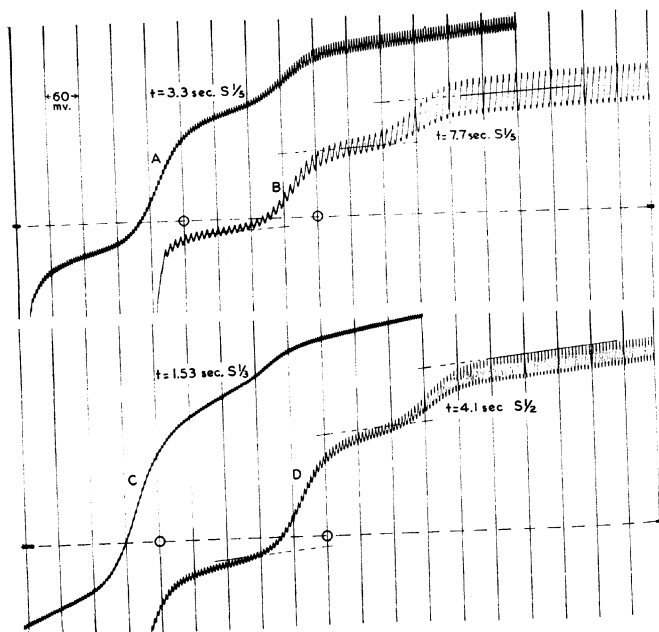


Fig. 13.—Polarograms of 0.0001 *M* α -oxyphenazine in 0.1 *N* H_2SO_4 obtained with capillaries of different drop times (*t*) (see text).⁹²

concentration and nature of the dye, on the temperature, and on the characteristics of the capillary. At a given dye concentration the suppression is always a definite fraction of the wave irrespective of the nature and concentration of the reversible system. Wiesner,^{92a} who discovered this phenomenon, feels that absorption of the dye is the cause of this "overvoltage" of reversible oxidation-reduction systems.

^{92a} K. Wiesner, *Collection Czechoslovak Chem. Commun.*, 12, 594 (1947).

TABLE IV

APPLICATION OF THE ILKOVIČ EQUATION TO THE DIFFUSION CURRENT OF α -OXYPHEN-AZINE MEASURED WITH DIFFERENT DROPPING MERCURY ELECTRODES AND USING DIFFERENT METHODS OF MEASUREMENT

Curve in Fig. 13	Capillary No.	κ	P, cm.	t, sec.	$m^{2/3}t^{1/2}/s$ mg./s. \times sec. $^{-1/2}$	Recorded galvanometer deflection					
						Average		Maximum		% of total	
						I_d $\times 10^{-8}$ amp.	I_d $m^{2/3}t^{1/2}/s$	I_d $\times 10^{-8}$ amp.	I_d $m^{2/3}t^{1/2}/s$	I_{d0} $\times 10^{-8}$ amp.	I_d $m^{2/3}t^{1/2}/s$
A	1	29.7	64	3.3	2.042	55.6	27.2	56.6	27.7	56.3	27.6
B	1	29.7	29	7.7	1.380	36.4	26.4	40.9	29.6	39.6	28.7
C	2	44.6	74	1.53	1.506	39.1	26.0	39.2	26.1	39.2	26.1
D	3	182.0	83	4.1	0.748	20.1	26.9	20.7	27.7	20.5	27.4
AVERAGE:						26.6		27.8		27.4	

D. REVERSIBLE OXIDATIONS AND REDUCTIONS IN UNBUFFERED OR POORLY BUFFERED SOLUTIONS

A consideration of the potential equation (24) of organic substances makes it obvious that the half-wave potential is only significant if the pH at the interface is known and constant, which means that adequate buffering is essential. It is therefore of practical importance, as well as of theoretical interest to be able to recognize the effect of inadequate buffering on the shape of the current-voltage curve.

The effect of buffering on the current-voltage curves of 0.0012 *M* solutions of quinhedrone⁹³ is illustrated in figure 14. In a solution of 0.00001 *M* phosphate in 0.1 *N* potassium nitrate (Curve *E*) which may be considered unbuffered, there are two separate waves, one anodic and one cathodic, with half-wave potentials indicating pH values far removed from that expected in a neutral solution. At higher concentrations of the pH 6.9 phosphate buffer, a third wave is observed between the two unbuffered waves which increases at the expense of the latter as more buffer is added (Curves *D* and *C*). It finally produces the continuous anodic-cathodic wave observed in well-buffered solutions (Curve *A*) when the buffer concentration is well in excess of the quinhedrone concentration. Additional experiments demonstrated that the half-wave potentials of the anodic waves become more positive, and those of the cathodic waves more negative, as the concentration of quinhedrone is increased in the unbuffered solutions. Müller^{68, 71, 93} explained these results as follows: During the reduction of quinone to hydroquinone (represented by the cathodic wave), hydrogen ions in an unbuffered solution must come from the surrounding water, thus leaving free hydroxyl ions and increasing the pH at the interface. On the

⁹³ O. H. Müller, *J. Am. Chem. Soc.*, **62**, 2434 (1940).

other hand, during the oxidation of hydroquinone to quinone (represented by the anodic wave), hydrogen ions are set free which, in the unbuffered solution, remain long enough at the electrode interface to produce a lower pH .

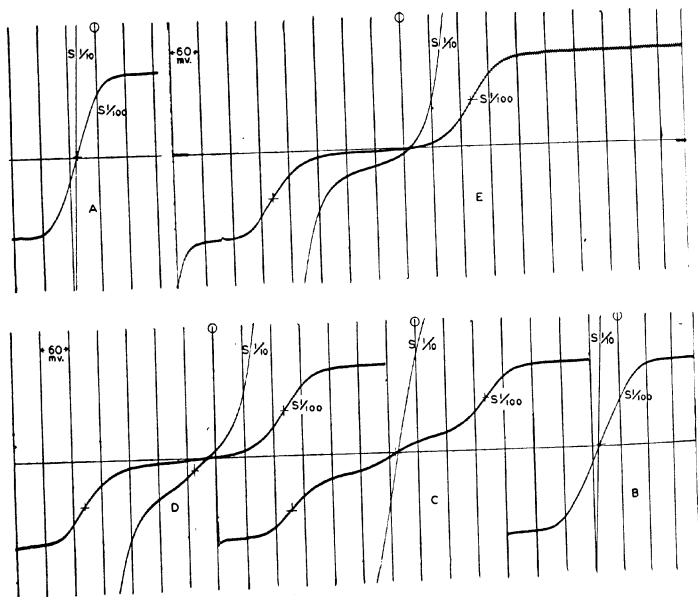


Fig. 14.—Polarograms of 0.0012 M solutions of quinhydrone in phosphate buffer of pH 6.9: (A) 0.1 M ; (B) 0.01 M ; (C) 0.001 M ; (D) 0.0001 M ; and (E) 0.00001 M . The indifferent electrolyte is 0.1 N potassium nitrate.⁹³ The curves taken at S'_{10} magnify the middle portions of the curves taken at S'_{100} .

This conclusion was further tested with hydroquinone⁷¹ and the following equation was derived which fitted the experimental data:

$$E = E_0^* + \frac{RT}{2F} \ln \left(\frac{-I}{I - I_{da}} \right) + \frac{RT}{F} \ln \frac{2I}{I_{da}} \quad (32)$$

In this equation, the dissociation constants of the reductant have been neglected and the hydrogen-ion concentration has been considered equal to twice the concentration of oxidant formed at the interface. E_0^* is dependent on the concentration of the reductant; it was defined as the E_0' of a system at a hydrogen-ion concentration equal to its own concentration. The second term describes the normal symmetrical S curve at constant pH ,

while the third term describes the shifts of points of this curve with changing pH.

Additional investigation showed that a constant discrepancy existed between the pH values calculated from the half-wave potentials and the pH values expected on the basis of concentration of the reactant. This is illustrated by the data given in table V, which were obtained with quinhydrone in unbuffered solutions.²³

The half-wave potentials of the separate anodic and cathodic waves were measured and corrected for IR. Then the corresponding pH was calculated on the basis of the known potential-pH relationship of the quinone-hydroquinone system, keeping in mind that the ratio of $[\text{Ox.}]/[\text{Red.}]$ is equal to $1/3$ for the anodic and equal to 3 for the cathodic wave at the unbuffered half-wave potentials. The results are given in the third column of table V. In the fourth column are shown the expected pH values, which were assumed equal to the negative logarithm of the quinhydrone concentration, except for the cathodic curves obtained at higher concentration, where suitable approximations were made for the ionization of the end product. It may be seen from the last column that the difference between the observed and expected pH values is consistently near 0.5 for the anodic and near 1.0 for the cathodic waves, regardless of concentration.

TABLE V

EFFECT OF QUINHYDRONE CONCENTRATION ON pH OF THE INTERFACE AT THE HALF-WAVE POTENTIAL IN UNBUFFERED SOLUTION*

Comparison between pH values observed and those expected if $E_{1/2} = E_0^*$ (Eq. 32)

Quinhydrone concentration, M	Wave	pH of interface at half-wave potential		Difference in pH units
		Observed	Expected	
0.000333	Anodic	5.0	4.5	0.5 ...
	Cathodic	8.6	9.5	... 1.1
0.00011	Anodic	4.5	4.0	0.5 ...
	Cathodic	9.0	10.0	... 1.0
0.000323	Anodic	4.0	3.5	0.5 ...
	Cathodic	9.4	10.4	... 1.0
0.001	Anodic	3.5	3.0	0.5 ...
	Cathodic	9.6	10.7	... 1.1
0.01	Anodic	2.6	2.0	0.6 ...
	Cathodic	10.2	11.3	... 1.1

* O. H. Müller, *J. Am. Chem. Soc.*, **62**, 2434 (1940).

According to Müller,²³ the most likely cause for the apparent loss in acidity and basicity is the diffusion of hydrogen and hydroxyl ions away from the electrode, since both have mobilities exceeding those of all other ions. On the basis of this hypothesis, the discrepancy found in the anodic waves should be about twice as large as that of the cathodic waves, while

actually the opposite is the case. The cause of these discrepancies was, therefore, still in doubt, until Kolthoff and Orlemann⁹¹ proved conclusively that, at least in the oxidation of hydroquinone, the above hypothesis was correct. By including the diffusion coefficient of hydrogen ions in their considerations, these authors were able to account quantitatively for the discrepancy of 0.5 pH units, found at the half-wave potential of the hydroquinone oxidation in unbuffered solution (Table V). Still unexplained is the abnormally high discrepancy in the case of the cathodic waves.

The modified equation (25), which holds for unbuffered *anodic* waves only, is:

$$E = E_0 + \frac{RT}{2F} \ln \frac{I_{dc} - I}{I - I_{da}} + \frac{RT}{F} \ln \frac{2I}{I_{da}} + \frac{RT}{F} \ln [\text{Red.}] + \frac{RT}{F} \ln \frac{D_{\text{Red.}}^{1/2}}{D_{\text{H}^+}^{1/2}} \quad (33)$$

From equation (33) are omitted: (a) the hydrogen-ion concentration of the body of the solution; (b) the dissociation constants, K_{a1} and K_{a2} ; and (c) the factor involving the ratio of the diffusion coefficients of oxidant and reductant, $RT/2F \ln D_{\text{Red.}}^{1/2}/D_{\text{Ox.}}^{1/2}$, because, in unbuffered anodic waves all these terms are negligibly small compared with the other factors of the equation.

It is obvious from the foregoing that adequate buffering is essential if a constant and significant half-wave potential is to be obtained in a reversible reaction at the dropping mercury electrode. This should be kept in mind whenever the constancy of the half-wave potential is doubted during changing concentration of the reacting material. It should also be remembered in interpreting the results of investigators who did not provide adequate buffering.

In general, a 100-fold concentration of buffer over that of the reacting material assures adequate buffering.⁶⁸ It can be calculated that, if the concentration of free hydrogen ions in the body of the solution is 100 times that of the reductant, the hydrogen-ion concentration at the interface, $[\text{H}^+]_i$, will be changed by 0.56% when the anodic diffusion current is reached. If the concentration ratio is equal to 10 only, $[\text{H}^+]_i$ will be changed by 5.6% under the same circumstances.

If the hydrogen-ion concentration is controlled by the action of a buffer, we must compare the concentration of the active components of the buffer, instead of the free hydrogen ions, with the concentration of the reactant. Only the salt of the buffer can remove hydrogen ions produced during an oxidation, while only the acid form of the buffer can furnish the hydrogen ions needed during a reduction. Obviously for best results, the buffer should be composed of equal parts of acid and salt, in other words, the pH

⁹¹ I. M. Kolthoff and E. F. Orlemann, *J. Am. Chem. Soc.*, **63**, 664 (1941).

should be equal to the pK_a of the buffer. Under these conditions, a buffer with a concentration 100 times that of the reactant will prevent changes of $[H^+]$, greater than 0.56% whether the curve is anodic or cathodic. This condition exists *e. g.*, when a 0.001 *M* solution of quinhydrone is polarographed in a 0.2 *N* acetate buffer of pH 4.6, in which the concentrations of acetic acid and sodium acetate are both 0.1 *N*. On the other hand, if this

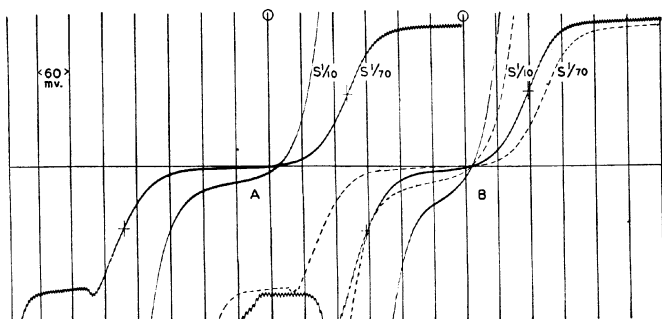


Fig. 15.—Polarograms of 0.001 *M* solutions of quinhydrone in: (A) 0.1 *N* potassium nitrate solution; (B) 0.01 *M* carbonate buffer of pH 7.5. Broken line is curve A superimposed on curve B.⁹³

solution were buffered at pH 3.6 (acetic acid = 0.18 *N*, sodium acetate = 0.02 *N*), the anodic wave would involve a change in $[H^+]$, of about 2.8% when the diffusion current is reached, while the cathodic wave would involve an even smaller pH change than before. The reverse would be the case if the solution were buffered at pH 5.6, where the acetic acid is 0.02 *N* and the sodium acetate is 0.18 *N*.

We have already seen the effects of small quantities of buffer added to an unbuffered solution of quinhydrone. The middle wave thus produced in curve C of figure 14 represents an *acid-base titration at the interface*, on which is superimposed a small change in the ratio of oxidant to reductant. Such waves can be evaluated; their midpoint corresponds to a pH equal to the pK_a of the buffer used. Within limits, such waves can also serve for the indirect quantitative analysis of the components of the buffer used.⁹⁵

The foregoing considerations imply that the diffusion coefficients of the components of the buffer are of the same order as those of the components of the oxidation-reduction system. Since equilibrium at the interface is established, the speed with which the buffers react to furnish or remove hydrogen ions must be as fast as the other electrode reactions. Most com-

⁹⁵ O. H. Müller, *unpublished results*.

mon buffers studied fulfill this condition, with the notable exception of the carbonate buffer.⁹³ As is demonstrated in figure 15, quinhydrone in a carbonate buffer gives separate anodic and cathodic waves (Curve *B*) similar to those found in an unbuffered solution (Curve *A*). Apparently some kind of buffer action must have taken place, because curve *A* cannot be superimposed on curve *B*, but it is decidedly different from the buffer action of a dilute solution of phosphate where a small middle wave is obtained (Curve *C*, Fig. 14), while the rest of the unbuffered waves remains practically unaltered. This action of the carbonate buffer is speeded up tremendously when a specific enzyme, carbonic anhydrase, is added to the solution. The polarogram then shows essentially the same single anodic-cathodic wave as in a phosphate buffer.^{95 a}

7. Influence of Temperature and Solvent on Half-Wave Potential

Since practical polarographic analysis is essentially a comparative method in which the medium and the temperature are kept constant in each case, very little has been done to investigate the effect of temperature and solvent on the half-wave potential. Nejedlý⁹⁶ found that the deposition potentials (45° tangent method) of several metal ions change by 0.1–1 mv. per degree rise in temperature, becoming more positive in the case of lead, indium, zinc, iron, and manganese ions and more negative in the case of thallium ions. This temperature coefficient is so small that it can be neglected for most practical purposes.

No such studies have been published for the half-wave potentials of organic compounds, although there would seem to be greater need for them in this field, because often the solubility and lability of the compounds investigated may demand the use of unusual temperatures.⁹⁴ For some *reversible* systems, the temperature coefficient of the half-wave potential may be anticipated from potentiometric work. As an example, the potential of quinhydrone is generally given as:

$$E_h \text{ (or } E_{1/2}) = 0.7177 - 0.00074t$$

when *t* is measured in degrees Centigrade. For the essentially *irreversible* reduction of pyruvic acid at the dropping mercury electrode, the writer found a similar negative temperature coefficient of 0.7 mv. per degree.⁹⁵ The influence of temperature becomes of greater importance when compounds are studied which can form semiquinones.⁹⁷

Of further interest in organic polarography is the effect of solvent on the

^{95a} O. H. Müller, *Federation Proc.*, **7**, 83 (1948).

⁹⁴ V. Nejedlý, *Collection Czechoslovak Chem. Commun.*, **1**, 319 (1929).

⁹⁷ O. H. Müller, *Trans. Electrochem. Soc.*, **87**, 441 (1945).

half-wave potential. This effect can again be anticipated on the basis of potentiometric work. For instance, Conant and Fieser⁹⁸ found the potential of quinhydrone at pH zero to be 0.699 v. in aqueous solution and 0.711 v. in 50% or 95% ethanol solution. Polarographic results obtained in aqueous solution,⁷³ and in 50% methanol solution,⁹⁹ are in excellent agreement with these data. In case the reductions involve semiquinone formation, one might also expect a significant effect of the solvent, judging from potentiometric data of Burstein and Davidson.¹⁰⁰ According to Müller,⁹⁷ an increase in temperature or the addition of ethanol to the solution tends to eliminate the anomalous wave (see page 1830).

Most studies are based on the assumption that the reference electrode has a constant potential or that the liquid junction potential remains constant during the electrolysis when salt bridges are used. In aqueous solutions this assumption is probably justifiable, but in water-organic solvent mixtures it may involve considerable errors. Results obtained in the latter medium must therefore be analyzed with care and should generally be treated as empirical. This is more so the case when the analysis is carried out in a completely nonaqueous medium (*e. g.*, glacial acetic acid, methanol, etc.). Here, usually a large layer of mercury on the bottom of the electrolysis vessel is used as the reference electrode. Its potential is, therefore, nearly always unknown, and it is questionable whether it is constant throughout the analysis. Furthermore, the cell resistance in nonaqueous solutions is extremely large; accordingly, the *IR* correction becomes more uncertain. Under these circumstances, the distance of the dropping electrode from the anode is of considerable importance, and a marked change in the resistance takes place as this distance decreases during the growth of a mercury drop. Unfortunately, these complications have not been sufficiently appreciated by students of nonaqueous solutions, so that their work must be considered as preliminary.¹⁰¹ To make data obtained in such studies of greater usefulness, it is urgently recommended that publications give the necessary information about the reference electrode, salt bridges used, if any, the cell resistance, and the accuracy with which it is measured.

8. Irreversible Reactions at the Dropping Mercury Electrode

Whenever it is impossible to oxidize (or reduce) the end product of a reaction (or oxidation) at the dropping mercury electrode at the same po-

⁹⁸ J. B. Conant and L. F. Fieser, *J. Am. Chem. Soc.*, **45**, 2194 (1923).

⁹⁹ L. I. Smith, I. M. Kolthoff, S. Wawzonek, and P. M. Ruoff, *J. Am. Chem. Soc.*, **63**, 1018 (1941).

¹⁰⁰ E. Burstein and A. W. Davidson, *Trans. Electrochem. Soc.*, **80**, 175 (1941).

¹⁰¹ G. B. Bachman and M. J. Astle, *J. Am. Chem. Soc.*, **64**, 1303, 2177 (1942).

tential at which it was formed, the reaction must be considered irreversible. On the basis of this criterion, we find that by far the majority of reactions of organic compounds which have been studied polarographically belong to this group. In fact, up to the investigation of quinuhydrone by Müller and Baumberger, in 1937, all polarographic reductions of organic compounds studied were irreversible. Since such reactions are considerably more complicated than reversible ones, the pioneer work of Heyrovský, Shikata, and Semerano remained largely empirical. At present, our knowledge is still inadequate for a thorough theoretical treatment of this topic; but with the information obtained with reversible systems, we are now considerably better equipped to analyze polarographic curves representing irreversible reactions and to speculate about the reaction mechanisms involved.

Space does not permit a discussion of the many hypotheses and theories which have been advanced to account for irreversible electrode reactions. None of these has been generally applicable, nor has any one of them been universally accepted. For example, in the case of hydrogen overvoltage, *i. e.*, the irreversible reduction of hydrogen ions, at least six different theories may be found in the current literature.¹⁰²⁻¹⁰⁶ The following discussion is, therefore, abbreviated and somewhat arbitrary; it may illustrate a few of the working hypotheses and the difficulties involved in fitting them to experimental data.

Preliminary to a consideration of irreversible reactions, it should be pointed out that even the reversible reactions studied give us no adequate means of knowing the actual reaction mechanism. Since we deal with equilibrium conditions in all these reversible reactions, we could have derived our final equations by considering the electrode primarily a hydrogen or an oxygen electrode (see, for example, Clark and Cohen⁸²). However, because it represents the most convenient and the most probable mechanism, we have developed our equations on the assumption that reversible electrode reactions of organic compounds involve primarily a transfer of electrons between the electrode and the organic substance, which is followed secondarily by associations or dissociations, all proceeding at rates sufficiently fast to maintain equilibrium conditions at the dropping mercury electrode.

We can then postulate that irreversible reactions fall into two main groups: (1) Those similar to the reversible reactions¹⁰⁷ involving a direct

¹⁰² T. Erdey-Grúz and M. Volmer, *Z. physik. Chem.*, **A150**, 203 (1930).

¹⁰³ R. W. Gurney, *Proc. Roy. Soc.*, **A134**, 137 (1931).

¹⁰⁴ A. Frumkin, *Z. physik. Chem.*, **A164**, 121 (1933).

¹⁰⁵ J. Heyrovský, *Collection Czechoslovak Chem. Commun.*, **9**, 273 (1937).

¹⁰⁶ H. Eyring, S. Glasstone, and K. J. Laidler, *Trans. Electrochem. Soc.*, **76**, 331 (1939).

¹⁰⁷ Some authors prefer to consider these as a special case of reversible reactions.

transfer of electrons between electrode and organic compound, but complicated (a) by a slow establishment of equilibrium conditions, or (b) by an irreversible further reaction (instability) of the end product. (2) Those differing from reversible reactions in the primary step, involving the reduction¹⁰⁸ of hydrogen ions to atomic or molecular hydrogen, which then reacts irreversibly with the organic compound (a) directly, or (b) after being catalytically activated by adsorption on the electrode material. There may, of course, be still other types of mechanism; for instance, a substance may be made electroactive by other agents present in solution which act as catalysts.

No comprehensive analysis of these possibilities has yet been made; only isolated examples can be given here to illustrate the complexity of irreversible reactions and the difficulties involved in their study. We may anticipate, however, that our first group of mechanisms will give rise to current-voltage curves which are very similar to reversible reactions, while the second group should produce curves with greater deviations from reversibility, especially since the reduction of hydrogen ions at the dropping mercury electrode is itself an irreversible process (see page 1851). We may rule out the possibility of hydrogen adsorption when dealing with a pure surface of mercury, but we should consider it whenever an organic reduction is preceded by reduction of a metal ion, since the deposited metal (which may be amalgamated or not) may have catalytic properties. Our problem in the future will be to analyze the polarographic waves as carefully as possible and ascertain any deviation from the known reversible ones. With sufficient data and by inclusion of proper rate constants, it may then be possible to derive suitable equations for the different irreversible reactions, and perhaps thus obtain information about the reaction mechanisms.

In the following discussion, we shall not consider cases in which the solution is unbuffered or in which the buffer is incapable of maintaining a constant pH at the electrode interface (see page 1838). Obviously, in all irreversible reactions involving hydrogen ions, adequate buffering is as essential as in reversible ones. Failure to recognize this fact has led to errors of interpretation on numerous occasions, as was pointed out by Müller.⁶⁸

A major difficulty in discussing irreversible reactions is that, in most of them, the end products are not known with any certainty, since not enough material is formed at the dropping mercury electrode to permit an analysis. Sometimes a reasonable guess can be made on the basis of results obtained when the electrolysis is carried out over a long period of

¹⁰⁸ The oxidation of hydroxyl ions need not be considered, since potentials positive enough for this reaction cannot be attained at the dropping mercury electrode.

time and with a large stationary electrode. However, the end products under these conditions are not necessarily identical with those formed at the dropping mercury electrode, and any generalization must be made with reservation. Even under relatively ideal conditions, different conclusions

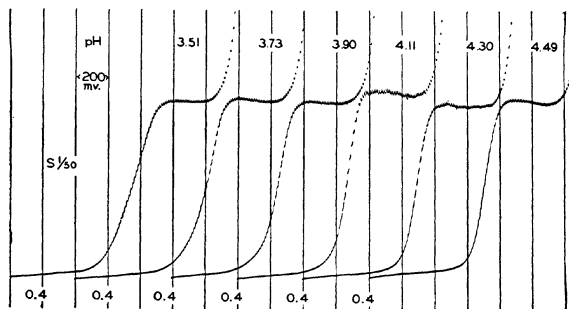


Fig. 16.—Polarogram of 0.001 *M* pyruvic acid reduced at different pH values.¹¹ Note that the waves in the more acid solutions are much less steep and lose their symmetry with respect to the midpoint, which also ceases to be the point of inflection of the waves.

may be reached. For instance, Semerano and Bettinelli¹⁰⁹ electrolyzed a few milligrams of aconitic acid at a dropping mercury electrode for about two weeks and then determined succinic acid fluorimetrically as one of the end products. On the other hand, Siebert,¹¹⁰ using multiple mercury drops (formed by letting mercury flow through a glass filter plate), electrolyzed 175 mg. of aconitic acid for 40 hours under otherwise supposedly identical conditions, and obtained tricarballic acid as the end product, with a yield of 97%. In some instances, one may predict a likely end product from the number of electrons involved in the reaction of a compound. To estimate this number, use is made of the Ilkovič equation in which an appropriate diffusion coefficient can be calculated or approximated.

Such a procedure is sometimes impossible because the relation between concentration and diffusion current is not always linear in irreversible reactions. Often, too, the half-wave potential fails to remain constant with changing concentration of the reactant. Here, and in cases in which the wave loses its symmetry, the half-wave potential becomes an arbitrary

¹⁰⁹ G. Semerano and G. Bettinelli, *Mem. accad. Italia, Classe sci. fis. mat. nat.*, **8**, 243 (1937). See also A. Miolati and G. Semerano, *Z. Elektrochem.*, **45**, 226 (1939).

¹¹⁰ H. Siebert, *Z. Elektrochem.*, **44**, 768 (1938); **45**, 228 (1939).

characteristic, as empirical as those discussed on page 1818. The polarogram of pyruvic acid, reproduced in figure 16 is a good illustration of a gradual change of a symmetrical into an assymetrical wave with change in the pH of the medium.⁷¹

In many cases, the half-wave potentials of irreversible reactions depend to a considerable degree on the concentration and kind of other electrolytes present in the solution. Especially higher valent cations have often shown a marked effect on the electrode reaction^{111, 112} and can certainly not be considered "indifferent," although they are discharged at more negative potentials than the organic compound. Such phenomena are especially striking in the reduction of inorganic anions.^{113, 114}

A. IRREVERSIBLE REACTIONS INVOLVING AN APPARENTLY REVERSIBLE STEP

Many irreversible reductions at the dropping mercury electrode give rise to symmetrical S-shaped waves with half-wave potentials that are a func-

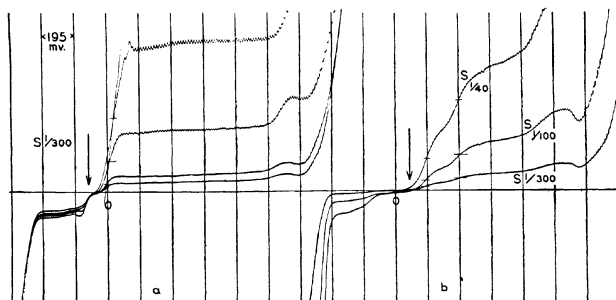


Fig. 17.—Polarograms of (a) azobenzene (about 4, 8, 33, and $84 \times 10^{-4} M$), and (b) dinitrobenzene (about $4 \times 10^{-4} M$) in 75% acetone and 0.2 N nitric acid.

Note graphic correction of the half-wave potentials of azobenzene for IR (see page 1877). In each instance, the value of Conant's A.R.P., represented by an arrow, is at the beginning of the rise in current and not at the half-wave potential.⁶⁸

tion of pH . To account for this type of current-voltage curve, Müller and Baumberger¹¹⁵ suggested that the polarographic wave represents a reversible step in the otherwise irreversible reductions. This made possible an

¹¹¹ E. Vopička, *Collection Czechoslovak Chem. Commun.*, **8**, 349 (1936).

¹¹² N. H. Furman and C. E. Bricker, *J. Am. Chem. Soc.*, **64**, 660 (1942).

¹¹³ M. Tokuoaka, *Collection Czechoslovak Chem. Commun.*, **4**, 444 (1932).

¹¹⁴ M. Tokuoaka and J. Růžička, *Collection Czechoslovak Chem. Commun.*, **6**, 339 (1934).

¹¹⁵ O. H. Müller and J. P. Baumberger, *J. Am. Chem. Soc.*, **61**, 590 (1939).

analogy with some irreversible reactions, investigated by Conant¹¹⁶ with an entirely different technique, in which a reversible step was similarly postulated. Less than half the compounds studied by Conant have been investigated polarographically, but the results uniformly indicate that Conant's "apparent reduction potential" (A.R.P.) coincides approximately with the beginning of the rise in current on the polarogram and not with the half-wave potentials. This is illustrated by the polarograms of azobenzene and dinitrobenzene⁶⁸ reproduced in figure 17. These results could be expected considering the methods of measurement; while Conant measured the A.R.P. indirectly by observing the slow irreversible process, the polarograph appears to record selectively the reversible portion of the reaction. Further verification of this point by additional evidence and complete analysis of the waves (derivation of the equation) would be desirable.

In the instances just mentioned, the irreversibly formed end product is no longer electroactive. A better insight into the reaction mechanism is obtained in the oxidation of hydroxychromans and hydroxycoumarans, where the end products are electroactive.⁹⁹ The oxidation products are quinones which can be reversibly reduced to the corresponding hydroquinones but not to the original hydroxychromans or hydroxycoumarans. The counterpart to these oxidations is the reduction of the dye, T-1824, also called Evans blue, which involves a reversible step followed by an irreversible transformation to a different but reversible system.¹¹⁷

B. SLUGGISH SYSTEMS

The reaction of quinhydrone in a carbonate buffer, discussed on page 1838, has demonstrated the existence of an acid which cannot react fast enough to maintain a constant pH during the electrode reaction. From this we may conclude that there may be oxidation-reduction systems consisting of acids or bases which may be equally slow in dissociating or associating. This slowness, of course, is only relative to the extremely fast rate of an electron transfer and would not be noticeable in an acid-base titration. Such an oxidation-reduction system would then appear to be irreversible; even in an adequately buffered solution the anodic and cathodic waves would not have the same half-wave potentials.

We may speculate that the so-called "sluggish" systems of potentiometry are of this nature. A number of biological oxidation-reduction systems appear perfectly reversible but require long periods of time to establish a significant potential at an indicator electrode. The potenti-

¹¹⁶ J. B. Conant, *Chem. Revs.*, **3**, 1 (1926).

¹¹⁷ H. Gilder, O. H. Müller, and R. A. Phillips, *Am. J. Physiol.*, **129**, 362 (1940).

metric study of such systems has been improved and simplified by adding to the solution a trace of a known electroactive oxidation-reduction system of suitable normal potential which acts as "mediator" and poises the electrode potential. Polarographic studies of such systems have so far made little progress; observations made with ascorbic acid, a typical sluggish system, illustrate some of the difficulties.¹¹⁸

C. STEPWISE IRREVERSIBLE REACTIONS

Under suitable conditions, it is often possible to observe stepwise reactions at the dropping mercury electrode even in irreversible reactions. Similar to the observations made in the case of semiquinones, the separa-

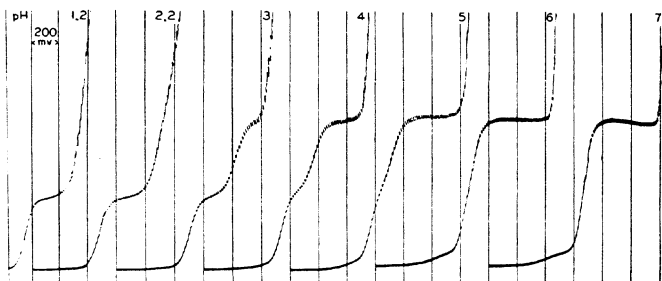


Fig. 18.—Polarogram of 0.005 *M* benzaldehyde, reduced at different pH values.¹¹⁹ Curves are recorded open to air at a sensitivity of $1/_{830}$ and begin at an applied voltage of -0.6 v.

tion of a single wave into equal steps depends very much on the pH of the solution. This is illustrated by figure 18, showing the reduction of benzaldehyde at different pH values.¹¹⁹ At pH 1.2 and 2.2, the second step can no longer be seen because it overlaps with the hydrogen wave. The product formed by the first step in acid solution was assumed to be hydrobenzoin, that obtained after the second step, benzyl alcohol.

The simple ratio in these stepwise reductions need not necessarily be unity in all cases. This may be demonstrated by figure 19, representing the reduction of picric acid¹²⁰ from well-buffered solutions over the range of pH 2.2 to 8. In the more acid solutions, two waves are obtained with a relative height of 3:2. The actual number of electrons involved in the

¹¹⁸ O. H. Müller and R. A. Phillips, *Am. J. Physiol.*, **129**, 426 (1940).

¹¹⁹ M. Tokuoka, *Collection Czechoslovak Chem. Commun.*, **7**, 392 (1935).

¹²⁰ O. H. Müller, *Cold Spring Harbor Symposia Quant. Biol.*, **7**, 59 (1939).

over-all reaction has been estimated by Lingane from *coulometric analysis*¹²¹ to be 17; the two steps therefore represent changes involving 10 and 7 electrons, respectively. The first large wave breaks up into two components of fixed proportions as the solution becomes more alkaline than pH 4, so that the reduction at pH 8 goes on in three steps with a ratio of 1:2:2 (involving 3, 7, and 7 electrons, respectively). Similar results have been observed with other nitrated phenols (see also the reduction of dinitrobenzene, figure 17, which goes on in two steps).

These reductions in steps of fixed ratio should not be confused with other reductions producing more than one wave on the polarogram which

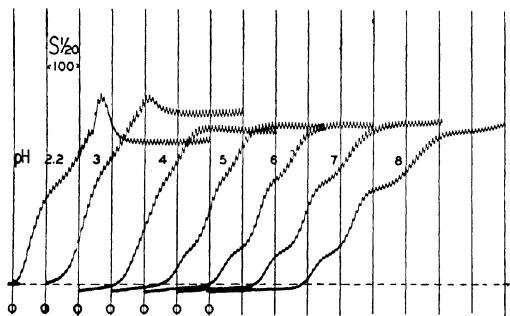


Fig. 19.—Polarogram showing step formation in the irreversible reduction of $2.1 \times 10^{-4} M$ picric acid with change in pH.¹²⁰

bear no such relationship. In such instances, we undoubtedly deal with the separate reduction of the different forms in which a given compound may exist. These multiple waves may be due to a separate reduction of the molecular and ionic forms of an ionizable compound (see page 1850), or they may be caused by a change in the structure or by polymerization of the reacting substance, which is influenced by the pH of the solution. Figure 20 illustrates the change in the polarographic wave of 0.001 *M* solutions of pyruvic acid¹¹⁵ as the pH of the medium is altered. Note that the single wave, obtained at pH 4.35, gradually diminishes as the solution is made more alkaline, to be displaced by a second wave with a more negative half-wave potential. The sum of the two waves, of course, is a constant. Müller and Baumberger attributed the first wave to the keto and the second wave to the enol form of pyruvate, with the implication that the tautomerization must proceed very slowly compared to the electron trans-

¹²¹ J. J. Lingane, *J. Am. Chem. Soc.*, **67**, 1916 (1945).

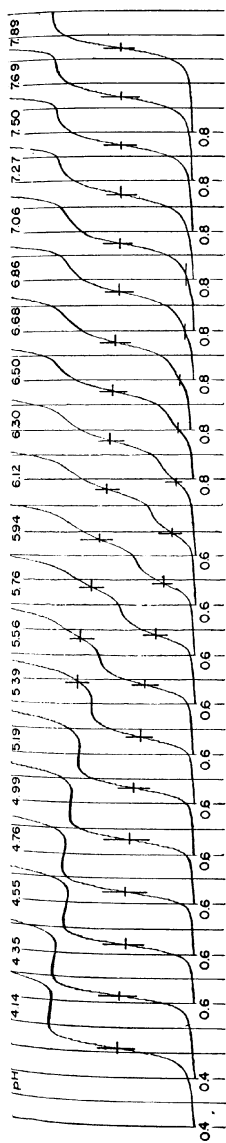


Fig. 20.—Current-voltage curves of 0.001 *M* pyruvic acid, buffered at the pH indicated, and demonstrating keto-enol tautomerism.¹¹⁵ The initial applied voltage for each curve is shown, the voltage increment is 200 mv. for each abscissa, and the sensitivity used is $1/50$.

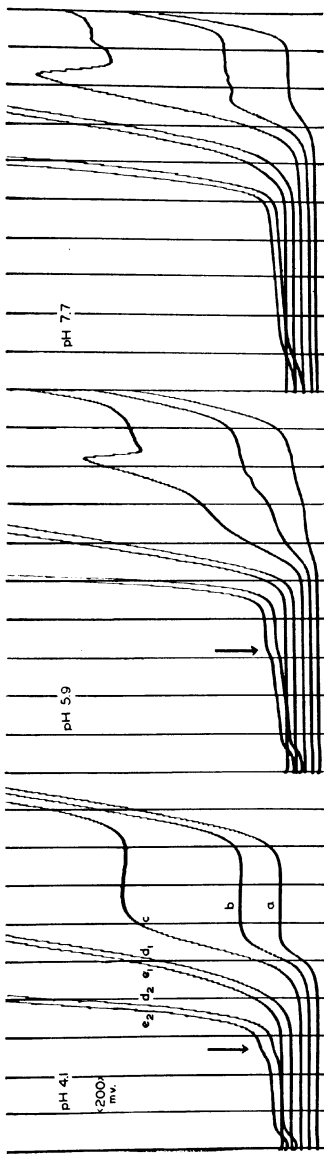


Fig. 21.—The polymerization of pyruvic acid as affected by pH and concentration.¹¹⁵ Three families of curves, all starting at zero applied voltage, with members at similar approximate concentrations and galvanometer sensitivities identically arranged as follows: (a) 0.001 *M*, (b) 0.002 *M*, (c) 0.005 *M*, (d₁) 0.1 *M*, and (e₂) 0.2 *M*; all at $1/500$ sensitivity; (d₂) 0.1 *M*, and (e₃) 0.2 *M*, each at $1/50$ sensitivity.

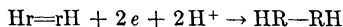
fer. If the tautomerization were very rapid, any enol pyruvate would be changed into the keto form as the latter is used up by the electric current, and only one wave would result.¹¹⁵ Since entirely analogous results were obtained with phenylglyoxylic acid, which cannot possibly enolize, Brdička¹²² concluded that the two waves are caused by the undissociated and dissociated forms of the two acids.

An effect of polymerization was found when the concentration of pyruvic acid was increased to 0.1 *M*. Figure 21 shows that, in the more acid solutions, a small wave (only observable at higher galvanometer sensitivities) appears which is absent in the alkaline solution. This new wave was ascribed to a polymer,¹¹⁵ the formation of which is favored in acid solution. From an analysis of similar curves, it was possible to follow the degree of polymerization with changes in pH and to estimate *n*, the number of molecules of pyruvic acid in the polymer. Since this involved the assumption of identical diffusion coefficients for polymer and monomer, it was only an approximation.

D. INFLUENCE OF IONIZATION AT POINTS UNCONCERNED IN THE ELECTRODE REACTION

So far in the discussion we have treated only acidic and basic ionizations at points directly concerned in the electron transfer. However, there are some reactions in which both oxidant and reductant can ionize at places which are unconcerned in the electron transfer, and we must consider the possibility that the ionization constants of oxidant and reductant are different. If they are identical, we may neglect them, as Clark and Cohen¹²³ have pointed out for reversible systems; but if they are not, a significant change will result in the potential-*pH* curves.

This will become clear if we consider as oxidant an unsaturated dibasic acid which is irreversibly reduced to the corresponding saturated acid (reductant), with the consumption of two hydrogen ions and two electrons:



Here *r* differs from *R* by a hydrogen atom and does not involve a dissociation constant. We may therefore write the potential equation as follows:

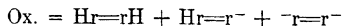
$$E = E_0 + RT/2F \ln [\text{ox.}]_i [\text{H}^+]_i^2 / [\text{red.}]_i \quad (34)$$

where [ox.] and [red.] are the concentrations of the electroactive portions of the oxidant and the reductant, which may be molecular or ionic. Since

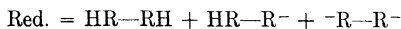
¹²² R. Brdička, *Chem. Listy*, **40**, 232 (1946); *Collection Czechoslovak Chem. Commun.* **12**, 212 (1947).

¹²³ W. M. Clark and B. Cohen, *U. S. Pub. Health Repts.*, **38**, 666 (1923).

their concentrations cannot be measured directly, we again use the capitalized summation terms, Ox. and Red., as on page 1826, where, in this case:



and:



As before, the concentration of the electroactive fraction can be defined by the total concentration of acid in all its forms and the ionization constants, K_o and K_r , of oxidant and reductant. Substitution in equation (34) then yields:

$$E = E_o + \frac{RT}{2F} \ln \frac{[\text{Ox.}]_i}{[\text{Red.}]_i} + \frac{RT}{F} \ln [\text{H}^+]_i + \frac{RT}{2F} \ln \frac{[\text{H}^+]_i^2 + K_{r1}[\text{H}^+]_i + K_{r1}K_{r2}}{[\text{H}^+]_i^2 + K_{o1}[\text{H}^+]_i + K_{o1}K_{o2}} \quad (35)$$

From this we see that if the K_r and K_o values are equal, the last term drops out; otherwise it should become of real significance.

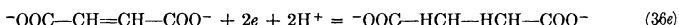
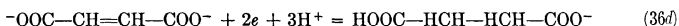
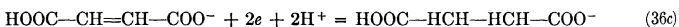
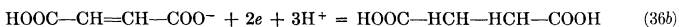
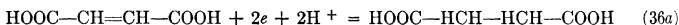
As a rule, the saturated acid is weaker than the unsaturated acid; hence we have numerous examples in the irreversible polarographic reduction of unsaturated acids in which the acid strengths of oxidant and reductant are different. This fact has, however, been almost universally neglected in polarographic studies, probably because they have been complicated by so many other major difficulties. As has been stated before, the end products in these reductions are uncertain and higher valent cations have a marked effect on the potential. In addition, the available data indicate that many of these acids ionize so slowly that the molecular form is reduced separately from the ionized forms, so that even in well-buffered solutions several waves are obtained which often overlap considerably. Furthermore, it is doubtful that the saturation of a double bond, which is unquestionably irreversible, is properly represented in equation (35). It seems likely that this process would necessitate a formulation similar to that needed in hydrogen overvoltage (see page 1851). In view of all these difficulties it is not surprising that equation (35) does not fit available experimental data unless it is considerably modified. Its purpose is merely to point out the significance of the dissociation constants of the oxidant and reductant. That this is real is illustrated by studies of Müller and Baumberger¹¹⁵ on pyruvic acid; the half-wave potential-pH curve showed an inflection near each of the pK_a values of pyruvic acid (the oxidant) and lactic acid (the reductant). On the other hand, attention should be called to an equation developed by Herasymenko¹²⁴ in which the dissociation of the reductant

¹²⁴ P. Herasymenko, *Collection Czechoslovak Chem. Commun.*, **9**, 104 (1937).

was completely neglected. This equation fits some of Vopička's data¹¹¹ on fumaric and maleic acids in very acid solutions; whether this is mere chance or of real significance must be established by further experiments.

Separate reductions of acid molecules and anions have been demonstrated by Schwaer,¹²⁵ Vopička,¹¹¹ and Brdička.¹²² Furman and Bricker¹¹² calculated on the basis of dissociation constants how much *o*-phthalic acid should exist in the molecular form, and obtained values in good agreement therewith directly from the polarographic curves. This would have been impossible had the dissociation or association of the acid been as fast as in the reversible systems described earlier in this article.

These results imply, of course, that in such instances a number of possible reactions must be considered. Taking, for example, the reduction of fumaric acid ($pK_{a_1} = 3.0$, $pK_{a_2} = 4.5$) to succinic acid ($pK_{a_1} = 4.0$, $pK_{a_2} = 5.3$), and assuming that fumaric acid ionizes extremely slowly (compared to the rate of the electron transfer and to the rate at which succinic acid ionizes), we may expect that, at different pH values, each of the following reactions gives rise to a separate wave:



Of these reactions, (b) and (d) will drop out if the ionization of succinic acid is as slow as that of fumaric acid. The pH at which these reactions occur and the clearness with which the individual waves can be observed, naturally depend to a large degree on the slowness of the ionization. In general, one may expect considerable overlapping, especially if the concentration of fumaric acid is fairly high. Published polarograms are not available to test these conclusions, but preliminary experiments of the writer⁹⁵ make them seem very probable.

In a theoretical treatise of the separate reduction of the undissociated and ionized forms of an acid, Brdička and Wiesner¹²⁶ derived equations which express it quantitatively and as a function of the pH of the solutions and the pK of the acid. On the basis of these equations, it should be possible to calculate the velocity constant, k , of the reaction $\text{A}^- + \text{H}^+ \rightarrow \text{HA}$, or the product μk , where μ represents the thickness of the interfacial layer in which the reaction takes place. These authors further believe that the dissociation constant of such acids could be determined from the

¹²⁵ L. Schwaer, *Collection Czechoslovak Chem. Commun.*, **7**, 326 (1935).

¹²⁶ R. Brdička and K. Wiesner, *Collection Czechoslovak Chem. Commun.*, **12**, 138 (1947).

change in the limiting currents resulting from an altered rate of flow of mercury through the capillary.¹²⁶

E. REDUCTION OF HYDROGEN IONS

Many, if not most, polarographic studies would be impossible were it not for the fact that the reduction of hydrogen ions at the dropping mercury electrode is irreversible, and takes place at a potential far more negative than that of the reversible hydrogen electrode. This difference in potential is called the overvoltage of hydrogen; it varies from metal to metal and seems to be largest at a fresh surface of mercury. A detailed review of this important problem and the many different theories advanced to explain it is beyond the scope of this discussion. A rough outline of the work done in polarography appears necessary, however, since we have often referred to the hydrogen overvoltage and have postulated that some irreversible reductions of organic compounds may proceed by way of hydrogen atoms formed by the reduction of hydrogen ions.

Heyrovský *et al.*¹²⁷ have made extensive studies of the reduction of hydrogen ions at the dropping mercury electrode and have established certain facts which must be considered in any theory of hydrogen overvoltage. These may best be stated by the empirical equation:

$$E = 2RT/F \ln [H^+]_e - RT/\beta F \ln I + A \quad (37)$$

The first term in this equation signifies that the shift in potential with a given change in hydrogen-ion concentration is twice that observed with a reversible hydrogen electrode. The second term describes the logarithmic increase of the current with an increase in voltage. The factor β varies, depending on the drop time and the current strength. In general, it is equal to 1 when the current is small and approaches $1/2$ at currents close to the diffusion current, while on the average it is equal to $2/3$.

To account for these values of β , Heyrovský makes the following assumptions. The reduction of hydrogen ions to atoms (nascent hydrogen) is perfectly reversible, but the combining of two hydrogen atoms into a molecule is slow unless catalyzed. In the absence of a catalyst, the newly formed hydrogen atom first combines with a hydrogen ion; the speed of

¹²⁷ J. Heyrovský, *Trans. Faraday Soc.*, 19, 785 (1924); *Rec. trav. chim.*, 44, 499 (1925). J. Heyrovský, *A Polarographic Study of the Electrokinetic Phenomena of Adsorption, Electroreduction and Overpotential Displayed at the Dropping Mercury Cathode*, Hermann, Paris, 1934. J. Heyrovský, *Collection Czechoslovak Chem. Commun.*, 9, 273 (1937). J. Heyrovský and O. H. Müller, *ibid.*, 7, 281 (1935). P. Herasymenko, *Rec. trav. chim.*, 44, 503 (1925). P. Herasymenko and I. Šlendyk, *Z. physik. Chem.*, A149, 123 (1930). J. Tomáš, *Collection Czechoslovak Chem. Commun.*, 9, 150 (1937). J. Novák, *ibid.*, 9, 207 (1937).

this reaction thus depends on the hydrogen-ion concentration near the electrode. These H_2^+ ions are then discharged at the electrode to hydrogen molecules. The latter are adsorbed for some time on the electrode surface and diminish the area to which the H_2^+ ions can have access, thus exerting some influence on the rate of the reaction. With the aid of the Langmuir adsorption isotherm, Heyrovský accounted for this blocking effect of the hydrogen molecules and obtained the following equation:

$$E = 2RT/F \ln [\text{H}^+]_i - RT/F \ln I(1 + \omega I) + A \quad (38)$$

When I is very small, $I(1 + \omega I)$ is practically equal to I , while at higher currents ωI becomes much larger than 1, and $I(1 + \omega I)$ approaches the value ωI^2 . Heyrovský's equation (38), derived on theoretical grounds, fits surprisingly well the experimental facts expressed by the empirical equation (37); but this does not prove that the proposed mechanism is correct. Objections which can be raised to this thesis cannot be discussed here.

For our purposes it is important to remember that the equation for the hydrogen wave is extremely complicated, and that a similarly complicated equation for the cathodic wave of an organic compound may well signify that the reduction is proceeding by way of hydrogen atoms. The contrary is not necessarily true, since it is possible that organic compounds react instantaneously with any hydrogen atoms available and produce waves which fit a simpler equation.

Of practical value is the knowledge that the reduction of hydrogen ions is shifted 120 mv. toward negative potentials with a tenfold decrease in hydrogen-ion concentration, while the corresponding shift for most organic reactions is between 90 and 30 mv. Certain waves which overlap with the hydrogen wave in acid solutions may therefore become well defined at a higher pH (see Fig. 18). Of course, in well-buffered solutions, the hydrogen wave will set a definite limit to the potential range.¹²⁸ This limit is conveniently estimated by means of the following equation:

$$\text{limiting } E = - (1.000 + 0.120 \text{ pH}) \text{ v. (vs. S.C.E.)} \quad (39)$$

At -1.9 v., the reduction of the alkali metals sets a new limit to the potential range.

III. APPARATUS

For polarographic analysis, any apparatus will be suitable which permits polarizing a microelectrode to a desired potential and measuring the current flowing at that potential. A sensitive and properly shunted galvanometer and a potentiometer, together with a dropping mercury electrode and a

¹²⁸ O. H. Müller, *J. Chem. Education*, **18**, 320 (1941).

large calomel half-cell, are perfectly adequate. But much simpler as well as more elaborate equipment is available. In the following, some of the main types of apparatus will be described. The choice of apparatus will depend on the work for which it is intended.

1. Simple Manual Equipment

An inexpensive, simple, and yet versatile device for obtaining polarographic current-voltage curves has been described by Müller.¹²⁹ The circuit used is shown schematically in figure 22. Besides the cell, which will be discussed later (see page 1865), the apparatus consists of three units: (1) the source of electromotive force (e. m. f.), (2) the polarizing unit, and (3) the galvanometer and shunt.

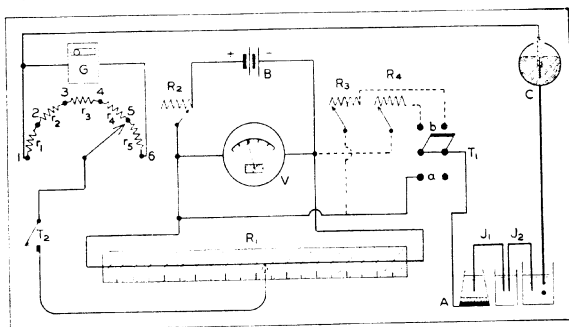


Fig. 22.—Circuit of simple manual equipment for obtaining current-voltage curves, including *normal* and *anodic* and *cathodic* polarizing units (see text).¹²⁹

Source of E. M. F.—A four- or six-volt storage battery, *B*, is best, but two or three normal dry cells in series can also be used.

Polarizing Unit.—In figure 22, two types of polarizing units are shown, either of which can be used by manipulation of the double-pole double-throw switch *T*₁. When switch *T*₁ is in position *a*, the *normal* polarizing unit is connected. It consists of a voltmeter, *V*, slide-wire *R*₁, tap key or switch *T*₂, and an additional resistance, *R*₂. The 3- or 5-v. voltmeter is connected across the ends of slide-wire *R*₁, and indicates the drop in potential across this wire. *R*₁ can be constructed from a meter stick and a No. 29 nichrome wire (with a *uniform* resistance of 5.28 ohms per foot), with a sliding contact made of a strong spring, supported by a brass arm. Good contact is assured all along the wire if it is cleaned with gasoline and

¹²⁹ O. H. Müller, *J. Chem. Education*, **18**, 111 (1941).

subsequently greased with a trace of vaseline. A commercial Wheatstone bridge with a similar total resistance can serve equally well. R_2 is a variable resistance of 100 ohms by means of which the voltage across R_1 can be set at some integral number of volts, indicated by voltmeter V . The two center posts of switch T_1 are connected with each other and to reference electrode A .

If switch T_1 is in position b , the *anodic and cathodic* polarization unit (see page 1794), shown by broken lines in figure 22, is connected. It adds to the *normal* polarizing unit two variable resistances, R_3 and R_4 , of 20 ohms each, which are in series. They are placed parallel to slide-wire R_1 and have a connection between them leading off to reference electrode A . If R_3 and R_4 are perfectly equal, the voltage applied from R_1 to the cell is zero when the slide makes contact at the 50-cm. mark; above 50 cm. the dropping mercury, C , is negatively polarized and below 50 cm. it is positively polarized. Thus, without interruption, a decreasing "anodic" polarization is followed by an increasing "cathodic" polarization. This feature is of little value in manually operated equipment, but is of real significance in automatic recording devices, when waves are studied near the potential of the reference electrode. A polarity-changing switch between the *normal* polarizing unit and the electrolysis cell may be found more convenient in the manual equipment.

For potential measurements of greater accuracy than is obtained with this simple equipment, a good potentiometer, which can be calibrated against a standard cell, should be used in place of the slide-wire.

The Galvanometer and Shunt.—Most desirable for polarographic work is a very sensitive galvanometer with a low internal resistance, a relatively high critical damping resistance, and a fairly long period of swing. For most purposes, any galvanometer can be recommended with characteristics similar to those of the *Nejedlý* moving coil galvanometer which has been used for the automatically recorded polarograms reproduced in this article. It has a sensitivity of 2.1×10^{-9} amp. per mm. per m., an internal resistance of 470 ohms, a critical damping resistance of 6000 ohms, and a half-period of swing of 8 seconds. The faster and cheaper *Leeds & Northrup Co.* galvanometer, which was used for the manually plotted polarograms (Figs. 4 and 9), also gives good results.¹³⁰ This is an enclosed lamp and scale-type galvanometer with a maximal sensitivity of 2.25×10^{-3} amp. per mm., a period of 3 seconds, an internal resistance of 1000 ohms, and a critical damping resistance of 15,000 ohms. Much faster galvanometers are not desirable, except for special studies, because they are too difficult to observe; if the galvanometer is too slow, its reading becomes too time-consuming.

¹³⁰ O. H. Müller, *J. Chem. Education*, 18, 172, 227 (1941).

The sensitivity of the galvanometer is regulated conveniently by means of an Ayrton shunt, which should have a total resistance equal to the critical damping resistance of the galvanometer. A galvanometer is critically damped when it indicates the flow of current in as short a time as possible for the type of galvanometer used without overshooting its mark. Obviously, in polarography, where the galvanometer is used to measure currents and not as a null instrument, it should be critically damped at all times. This is especially important in automatically recorded polarograms. The effect of the damping resistance may be visualized by remembering that a galvanometer, deflected from its zero position, will generate electricity in its coil while it is moving in the stationary magnetic field on its return to the zero position. A critical resistance or Ayrton shunt can then be found which, when placed across the terminals of the galvanometer, will slow down the movement of the coil just enough so that it will reach its zero position without overshooting. Regardless of the setting of the shunt, the galvanometer is then always critically damped.

The shunt can be a variable resistance as shown in figure 1 (page 1787), or a series of fixed resistances, r , as in figure 22. With the variable resistance, a galvanometer can be calibrated to read directly in per cent or in milligrams of a particular compound which is analyzed,¹³¹ but each new setting of the resistance requires a new calibration. The fixed resistances, on the other hand, if precision-made and properly chosen, make possible the selection of a suitable known fraction of the maximal sensitivity by the mere turning of a switch. Most convenient is a series of sensitivities, progressing as follows: 1/1, 2/3, 1/2, 1/3, 1/4, 1/5, 1/7, 1/10, 1/15, 1/20, etc., because then a sensitivity can be chosen to make the galvanometer deflections in a given case as large as the scale permits and thus effect more accurate measurements of the current. It is also possible to use less expensive resistors and calibrate the galvanometer sensitivities carefully at each setting of the shunt when the apparatus is first assembled.¹²⁹ These, of course, will not be in the simple ratios shown above. It must be remembered for purposes of calculating IR (page 1793) that the resistance of a shunted galvanometer varies with the setting of the shunt and that it reaches a maximum value when the galvanometer sensitivity is reduced one-half. Using fixed resistors one can calculate the shunt resistance at each setting and then make it equal to the maximum value by means of additional resistances.

The apparatus described is made up of standard equipment. It is adequate for all types of polarographic work, but for extended research, some commercially available, completely assembled, and compact manual instruments may be more desirable. One of these, the Eledropode (*Fisher Scientific Co.*, Pittsburgh, Pa.) has a circuit similar to the *normal* polarizing

¹³¹ B. P. Caldwell and S. Reznick, *Ind. Eng. Chem., Anal. Ed.*, **14**, 187 (1942).

unit described above. It has the advantages that a fairly large scale is used with the galvanometer and that all parts are completely enclosed in one single portable unit.

2. Automatic Recording Apparatus

The polarographic method has reached its present state of development largely through the invention of the automatic polarograph by Heyrovský and Shikata.¹³² This machine has been a tremendous timesaver; the records obtained with it have demonstrated more than anything else the unusually good reproducibility of the polarographic curves. The principle of the apparatus and the electrical circuit have been described in connection with figure 1 (page 1787).

Since its invention, the polarograph has undergone many changes in design, largely through the efforts of the manufacturers, *Dr. V. and J. Nejedlý*, Prague. For the past 15 years, the writer has used one of their standard models which has the following features: (1) The potentiometric wire, wound in 19 turns on the potentiometric wheel, can be calibrated against a Weston standard cell, and the potentials can be read accurately to $\pm 1\text{mv}$. (2) To avoid contact trouble, met in previous instruments, the ends of the potentiometric wire are firmly attached to leads which can rotate in two mercury pools connected to the battery circuit (Novák¹²⁷). (3) The electric motor which drives the potentiometric wheel is governor-controlled and fitted with reversing gears so that the voltage can be increased or decreased (forward or backward polarization, see page 1858). (4) With each full turn of the potentiometric wheel, the photographic drum is rotated 1 cm. past the slit in the camera, and light from a small lamp illuminates the whole slit for an instant, thus automatically plotting the voltage scale (abscissae). The size of the record is 10×20 cm. (5) A built-in clutch permits rotation of the photographic paper independently of the potentiometric wheel for the recording of current-time curves (see Fig. 30, page 1880). (6) Resistances are built into the apparatus for "anodic and cathodic polarization." (7) A scale on the camera and a mirror suitably placed make the instrument useful for direct observation as well as for recording. (8) With the camera housing removed and graph paper on the photographic drum, a rough outline of the galvanometer excursions can be traced with a pencil.¹³³ (9) All wiring and connections are readily accessible for trouble-shooting.

This polarograph is a very flexible recording instrument, suitable for many different types of research.¹³⁴ The construction of a very similar

¹³² J. Heyrovský and M. Shikata, *Rec. trav. chim.*, **44**, 496 (1925).

¹³³ H. Hohn, *Chemische Analysen mit dem Polarographen*. Springer, Berlin, 1937.

¹³⁴ O. H. Müller, *Ind. Eng. Chem., Anal. Ed.*, **14**, 99 (1942).

instrument from materials normally available in this country has been described by Furman, Bricker, and Whitesell,¹³⁵ who also discuss some of the complications which may be encountered in its operation.

Within the past 10 years some 20 different modifications of the polarograph have been described in the literature or have been advertised by manufacturers under a variety of names. It is not the purpose of this article to discuss all of them, but it seems worth while to point out some of the important differences among them. Most of them have been designed to provide industry with apparatus suitable for routine work in which many of the features found in the research type of polarographs have purposely been left out. To avoid the necessity of recording in semidarkness, the newer instruments have been completely enclosed. Whenever such designs contain a sensitive galvanometer (*e. g.*, the Micropolarograph and the Heyrovský Polarograph Model XII, *E. H. Sargent and Co.*, Chicago, Ill.), they require vibration-free mountings¹³⁶ to prevent irregular curves or damage to the galvanometer.

For those who object to the development of photographic records several instruments are available. Of these may be mentioned the Photo-pen Recorder (*National Technical Laboratories*, South Pasadena, Calif.) which records with ink on a roll of paper¹³⁷; it operates by keeping light reflected from a galvanometer centered between the two surfaces of a double-cathode phototube. The Electrochemograph (*Leeds and Northrup Co.*, Philadelphia, Pa.) is an adaptation to polarography of the Micromax recording potentiometer which records with ink on a roll of paper. This recording is done by means of a sensitive mechanism which periodically applies sufficient counter electromotive force to keep a galvanometer in zero position. The Sargent Model XXI Visible Recording Polarograph (*E. H. Sargent and Co.*, Chicago, Ill.) is a similar instrument with a continuously recording potentiometer recorder which acts like a critically damped galvanometer. In the high speed, visible recording Polaro-Analyzer (*Rutherford Instrument Co.*, Alexandria, Va.) the currents are amplified electronically. As these instruments increase in complexity so do they increase in price and become more difficult to repair. Yet, as was pointed out by Lingane¹³⁸ in a discussion of some of these instruments, "none of the recording instruments commercially available today is capable of yielding polarographic data of greater accuracy than one can obtain with relatively simple manual apparatus, and some of them are distinctly inferior to the manual equipment in

¹³⁵ N. H. Furman, C. E. Bricker, and E. B. Whitesell, *Ind. Eng. Chem., Anal. Ed.*, **14**, 333 (1942).

¹³⁶ G. E. Philbrook and H. M. Grubb, *Anal. Chem.*, **19**, 7 (1947).

¹³⁷ D. J. Pompeo and C. J. Penther, *Rev. Sci. Instruments*, **13**, 218 (1942).

¹³⁸ J. J. Lingane, *Ind. Eng. Chem., Anal. Ed.*, **18**, 734 (1946).

this respect. The sole advantage of a recording polarograph over manual instrumentation is its greater convenience."

All recording instruments have in common one disadvantage, namely, a certain lag between the applied voltage and the recorded current. The magnitude of this lag depends on the rate with which the applied voltage is increased and on the speed of the current-indicating device. In the standard polarograph, this lag is usually small and can be easily determined by first polarizing forward, *i. e.*, making the dropping mercury electrode more and more negative, and then reversing the process, *i. e.*, polarizing backward. The difference between the two half-wave potentials thus obtained is equal to twice the lag. Whenever this technique is not possible, one must prepare current-voltage curves of a solution containing two simple ions with known well-established half-wave potentials. After correction for IR , the difference between the two half-wave potentials, compared with the difference theoretically expected, will give an indication of the lag. To minimize this lag, the rate at which the applied voltage is increased should be slow. In general, the time required for the preparation of a polarogram varies from 2 to 20 minutes, depending on the voltage range covered and on the speed of the recording device.

3. Use of Electron Tubes for Polarographic Studies

Even automatic recording instruments are slow compared with the practically instantaneous devices in which electron tubes are used. Attempts to apply such devices to polarography have been very promising from the practical as well as the theoretical standpoint but can only be mentioned in this discussion since they are concerned largely with inorganic reactions. Three fundamentally different applications have been considered, all of which can be quantitative as well as qualitative: (1) the cathode-ray tube is used to obtain a current-voltage curve of a single drop of mercury, by synchronizing drop time and sweep frequency;¹³⁹ (2) either a cathode-ray tube or an electric eye is used merely as an indicator;¹⁴⁰ and (3) the cathode-ray tube is used to obtain potential-time curves at high frequencies.¹⁴¹ The latter technique has already yielded some new information about the rate and reversibility of different steps in organic electrode reactions. Differences in the results obtained in this way with the dropping mercury electrode and with the streaming mercury electrode (see p. 1882)

¹³⁹ L. A. Matheson and N. Nichols, *Trans. Electrochem. Soc.*, **73**, 193 (1938).

¹⁴⁰ R. H. Müller, R. L. Garman, M. E. Droz, and J. Petras, *Ind. Eng. Chem., Anal. Ed.*, **10**, 339 (1938). J. Boeke and H. van Suchtelen, *Z. Elektrochem.*, **45**, 753 (1939).

¹⁴¹ J. Heyrovský, *Chem. Listy*, **35**, 155 (1941) and **40**, 229 (1946). R. Bieber and G. Trümpler, *Helv. Chim. Acta*, **30**, 971 (1947). J. Heyrovský and J. Forejt, *Z. physik. Chem.*, **193**, 77 (1943).

have indicated a temporal separation of several stages in the reduction of nitrobenzene.

4. Dropping Mercury Electrode

The type of dropping mercury electrode used in polarography was originally introduced by Kučera¹⁴² for surface-tension measurements of polarized mercury where the weight of the drops was used as criterion. In its simplest form, it consists of a very narrow capillary connected by means of thick-walled rubber tubing to a mercury reservoir (leveling bulb) the height of which can be adjusted to produce a desired drop time. Contact between the Hg and the electrical circuit is made by means of Pt wire to prevent contamination of the electrode metal. The rubber tubing is about 60–70 cm. long and must be boiled first in a strong NaOH solution and then several times in distilled water to remove traces of sulfur and other impurities. After that, it is thoroughly dried overnight. Depending on the type of investigation, capillaries of widely differing characteristics are used. For general work, a capillary is recommended which will deliver in 3–6 seconds a drop of mercury weighing between 3 and 6 mg., although considerably faster electrodes have also given satisfactory results.

The capillaries may be prepared by anyone with some experience in glass blowing by drawing out a wider capillary following the directions of Heyrovský.¹⁴³ Good tips thus obtained should be about 2–4 cm. long with an internal diameter of 0.03–0.05 mm. and an external diameter greater than 1 mm. Since these capillaries are quite fragile, commercial thermometer tubing of very fine bore¹⁴⁴ is now more widely used. In microanalyses, of course, the former still offers an advantage because the latter would have to be ground to a smaller external diameter. After assembly, the dropping mercury electrode should be tested for performance by determining the two capillary constants as described on page 1813. Naturally, *the electrode must be mounted on a vibrationless stand so that the drops fall under their own weight.*

This simple construction of the dropping mercury electrode is also the most flexible and has been used for practically all the developmental work. Lately, a number of modifications of this construction have been proposed, most of them with the aim of substituting glass or plastic tubing which

¹⁴² G. Kučera, *Ann. Physik*, **11**, 529 (1903).

¹⁴³ J. Heyrovský, "Polarographie," in W. Böttger, *Physikalische Methoden der analytischen Chemie*. Vol. II, Akadem. Verlagsgesellschaft, Leipzig, 1936.

¹⁴⁴ The "marine barometer tubing" of the *Corning Glass Works*, Corning, N. Y., with an internal diameter of 0.05 mm. or less, cut to appropriate lengths, is very satisfactory in the construction of the dropping mercury electrode (I. M. Kolthoff and J. J. Lingane, *Polarography*, Interscience, New York, 1941).

contains no sulfur (*e. g.*, Tygon) for the rubber tubing. Details of such modifications cannot be given here, but it should be pointed out that additional glass makes the setup more fragile.

To simplify the measurement of the mercury pressure above the capillary tip, a glass stand-tube of narrow bore is frequently used.¹⁴⁵ The writer has found a discarded calibrated manometer tubing from a Van

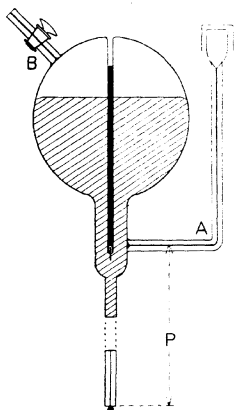


Fig. 23.—Mercury reservoir designed to keep the mercury pressure constant (see text).

Slyke apparatus very convenient for this. Small variations in pressure can then be easily recognized, or adjustments to known pressures can be made by raising or lowering the reservoir. (An excellent device for this purpose is the self-locking Castaloy leveling bulb support with fine adjustment, manufactured by *Fisher Scientific Co.*, Pittsburgh, Pa.) In some designs the mercury is forced into the stand-tube by air pressure from a rubber pressure bulb,¹³⁸ but there one must guard against air leaks during the experiment.

To assure a constant pressure on the capillary during routine analyses, the mercury leveling bulb can be modified as is shown in figure 23. This design¹⁴⁶ differs from the one described by Mueller¹⁴⁷ in that the level to which the mercury pressure is reduced is easily visible. With the stopcock *B* open, the reservoir is filled through capillary tube *A*; then gentle suction is applied at *B* until bubbles of air come through the mercury, at which point the stopcock is closed. A constant pressure, *P*, equal to the distance between the tip of the capillary and the junction of side tube *A* with the bulb, is guaranteed as long as no mercury is visible in the side tube.

In experiments in which light absorption is studied simultaneously with processes going on at the dropping mercury electrode,¹⁴⁸ or in which the capillary must be electrically shielded,¹⁴⁰ the design shown in figure 24 has proved very satisfactory because the whole unit can be easily enclosed in a suitable box. Here, the mercury reservoir is sealed directly to the capillary,

¹⁴⁵ J. J. Lingane and H. A. Laitinen, *Ind. Eng. Chem., Anal. Ed.*, **11**, 504 (1939).

¹⁴⁶ Designed by Dr. J. R. Rachele, of the Department of Biochemistry, *Cornell University Medical College*, New York City.

¹⁴⁷ E. F. Mueller, *Ind. Eng. Chem., Anal. Ed.*, **12**, 171 (1940).

¹⁴⁸ J. P. Baumberger and R. K. Skow, *Am. J. Physiol.*, **116**, 8 (1936). J. P. Baumberger, *ibid.*, **123**, 10 (1938).

and air pressure is applied to the top of the mercury from the outside. The total pressure of mercury is, of course, equal to the sum of the pressures in the small dropping mercury electrode unit (P_1) plus that indicated by the manometer (P_2).

In this and other all-glass apparatus, it is convenient to seal the capillary and reservoir to standard taper ground joints to permit easy exchange of capillaries.

The use of a *multiple dropping mercury electrode* (two or more electrodes joined together) may prove to have more disadvantages than advantages.^{148a}

A *knock-off electrode* has been designed in which the drops of mercury are knocked off mechanically or electrically.^{148b} This electrode may offer great advantages in differential polarography (see page 1873). It may be

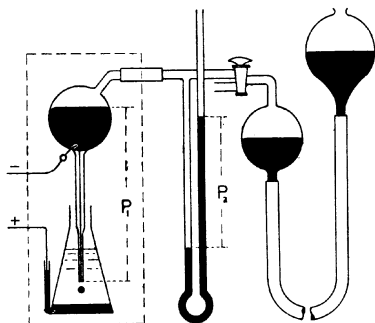


Fig. 24.—Compact dropping mercury electrode and cell suitable for *shielding* against light or electrostatic disturbances.

of even greater value generally, because it would eliminate complications arising from surface tension effects, especially the change in drop time which results from the altered surface tension as the voltage is increased (see page 1809). Unfortunately this knock-off electrode has not yet been tested sufficiently to insure its performance in these respects.

Replacement of a defective capillary with a new one not only involves the trouble and time necessary for its installation and calibration, but often necessitates an involved and not too accurate recalculation of the results obtained with the discarded capillary. It is, therefore, of paramount im-

^{148a} C. E. Bricker and N. H. Furman, *Anal. Chem.*, 20, 1123 (1948).

^{148b} E. D. Coleman, U. S. Patent 2,343,885 (1944). O. Kanner and E. D. Coleman, U. S. Patent 2,361,295 (1944).

portance to keep a capillary in good working order as long as possible. To do this, Heyrovský recommends, on the basis of many years of experience, that *the capillary remain dropping at all times unless it is immersed in distilled water*. The beginner usually does not appreciate this important direction until, through neglect of it, he has had to discard a few days' or weeks' work.

After an experiment, the capillary *while dropping* should be washed thoroughly with distilled water, cleaned off with filter paper, and then immersed in distilled water. Only then should the flow of mercury be stopped by lowering the mercury reservoir.¹⁴⁹ Since water or an aqueous solution wets glass, while mercury does not, some solution will always enter the tip of the capillary and should be washed out after conclusion of the experiment. Keeping the tip of the electrode in distilled water when stopping the flow of mercury will keep it washed out, and the capillary will remain in working order for many years, as has been found by actual experience. Sometimes, especially in studies of biological fluids, water is not enough to wash out the tip. According to the type of solution studied, the tip can then be cleaned, while the mercury is still flowing, by immersion in the proper organic solvent or in concentrated acid or alkali, followed by a rinse with water. It should then have the same capillary constants as when first assembled. If this is not the case, the dropping mercury electrode should be taken apart and the capillary cleaned more thoroughly. First *aqua regia*, then water, and finally filtered air is passed through the capillary; a blowing and sucking procedure is usually more effective than either one alone. Unless this cleaning process restores the original characteristics to the capillary, the latter must be discarded.

5. Purification of Mercury

It has always been stressed in the polarographic literature that only "purest" mercury be used for the dropping mercury electrode. Lacking a satisfactory criterion of this purity, experimenters "purified" their mercury regardless of its purity and described their procedure of purification rather than the degree of purity of the mercury. For instance, Heyrovský¹⁴³ recommended distillation in a current of air, preceded by chemical treatment of the mercury and followed by vacuum distillation. As Hulett and Minchin¹⁵⁰ have shown, vacuum distillation alone is not sufficient for the removal of base metals; even "triple distilled" mercury:

¹⁴⁹ From equation (10) it can be calculated that, as long as the capillary orifice is not larger than 0.05 mm. in diameter, the flow of mercury will stop in water when the mercury pressure is reduced to 22 cm. or less.

¹⁵⁰ G. A. Hulett and H. D. Minchin, *Phys. Rev.*, **21**, 388 (1905).

therefore, is satisfactory only if all base metals have been carefully removed before the vacuum distillation.

Many unnecessary "purifications" may thus have been carried out on mercury that was already free from any determinable impurities. This was suggested recently by Wichers,¹⁵¹ who described a simple test for the purity of mercury. For various reasons, even purest mercury may show a film or a dark ring on the glass in which it has been stored for a considerable time. However, when this film is removed by filtering the mercury through a pinhole in a paper cone or through a chamois, the pure can be distinguished from the impure mercury because the latter will form a new film or deposit on the glass almost immediately after it is filtered. Wichers¹⁵¹ states that impurities of base metals in the mercury in concentration of 1-2 parts per 10,000,000 could be detected by the formation of a film or "tailing" of the *dry* mercury.

Another test of similar sensitivity is the "foam test" described by Müller.¹⁵² If pure mercury is shaken with distilled water, a fairly stable foam is formed which disappears *gradually* in from 5-15 seconds. In contrast to this, very "dirty" mercury does not foam at all. Mercury which contains mere traces of impurities does foam when shaken with water, but the foam is stable for one or two seconds only and then collapses *suddenly*. It is essential for this test that the distilled water not contain any organic material and that the flasks used be scrupulously clean.

The foam test is of greatest value in combination with the following *procedure for purifying mercury for polarographic studies*. The used mercury is first shaken with water in a heavy-walled separatory funnel. If the mercury is very dirty, suction is applied to the top of the separatory funnel and a stream of air is passed through the mercury by regulating the stopcock. After several hours, the air will have oxidized all the base metals and floated them off as a thick scum in the water layer. The lower, cleanest portion of the mercury is then allowed to fall through the ungreased stopcock in a fine stream of small drops into a heavy-walled flask or separatory funnel, partially filled with a solution of nitric acid and mercurous nitrate. Next, the mercury is shaken vigorously with the acid for several minutes and drained through a separatory funnel—again in a stream of small drops—into a flask partially filled with distilled water. If some acid has come along with the mercury, a second washing with distilled water is necessary before a stable foam results when the mercury and water are shaken together. If no stable foam appears, further purification with nitric acid is indicated. The scum retained in the funnel after each separation is collected in a special flask for further treatment.

¹⁵¹ E. Wichers, *Chem. Eng. News*, 20, 1111 (1942).

¹⁵² O. H. Müller, *Chem. Eng. News*, 20, 1528 (1942).

The mercury which produces a good foam with water is poured through a pinhole in a cone of filter paper and can be completely squeezed through this pinhole without leaving any residue on the paper. Such mercury can be used directly for "pool" mercury anodes. Before the mercury is used for the dropping mercury electrode, however, it is usually purified further by vacuum distillation. This removes any noble metals which might lower the very high overvoltage of hydrogen observed on pure mercury, or which might act as catalysts for some organic reactions.

To guard against *mercury poisoning*, the greatest care should be taken not to spill any mercury on the desks or floor of the laboratory. A simple precaution is to have a large tray of water beneath the electrode stand so that any spilled mercury will immediately be covered with water. A short vigorous airing of the room at the start of work has been found very effective in reducing the mercury vapor in a room that has been closed overnight and has had floor cracks full of mercury.

6. Nonpolarizable Reference Electrodes

In early polarographic work carried out with inorganic solutions, a large pool of mercury at the bottom of the electrolysis vessel served as nonpolarizable anode, the potential of which was measured against a standard calomel electrode. The advantages were that the internal resistance of the cell remained small and that reduction products dissolved in the mercury drops were returned to the solution as soon as these drops merged with the anode. The effect of size and other factors on the constancy of such an electrode has been studied by Majer¹⁵³; as could be expected, the potential of the anode was most stable when the solution contained halide ions.

Instead of the anodic mercury, Schwarz¹⁵⁴ used a silver wire, coated with silver chloride, which was wound around the tip of the dropping mercury electrode. Since this reference electrode neither takes up any appreciable space nor requires special vessels, it is particularly well suited for microanalyses and routine work.

Since halide ions are usually absent when organic reactions are studied in a buffer solution, Müller and Baumberger¹⁵⁵ introduced the use of an external reference electrode of known and constant potential which is connected to the test solution in the electrolytic cell by means of an agar bridge containing a suitable electrolyte. This technique, which has been customary in potentiometric studies of organic oxidation-reduction systems, offers the advantage that all polarographic curves are referred to the same

¹⁵³ V. Majer, *Collection Czechoslovak Chem. Commun.*, **7**, 146 (1935).

¹⁵⁴ K. Schwarz, *Z. anal. Chem.*, **115**, 161 (1939).

¹⁵⁵ O. H. Müller and J. P. Baumberger, *Trans. Electrochem. Soc.*, **71**, 169 (1937).

reference electrode and thus can be compared directly. A further practical advantage is that much less mercury is needed for the measurements.

The most widely used reference electrode is the saturated calomel half-cell, but other standard half-cells are equally satisfactory. Of these, the hydrogen electrode is especially convenient to use because its potential can be varied at will by the experimenter, simply by using different buffers.¹⁵⁶

The *agar bridges* are usually saturated with potassium chloride to minimize the liquid junction potential. However, such bridges are not suitable when potentials more positive than $E_h + 0.250$ v. are studied, since chloride ions interfere.¹⁵⁵ In this case, it is best to work with two bridges, J_1 and J_2 , as shown in figure 22. Bridge J_1 , a potassium chloride bridge, preserves the calomel half-cell from contamination and leads to a junction vessel containing saturated potassium chloride, which can be replaced frequently. This is connected to the test solution by a second bridge, J_2 , which may be fitted with ground-glass plugs at either end and filled with the test solution, or may simply be a potassium nitrate agar bridge.

Agar bridges may be prepared by warming in a water bath a suspension of 3 g. of powdered agar-agar in 100 ml. of either saturated potassium chloride or 13% potassium nitrate solution. When the solution is homogeneous, it is sucked into glass U tubes of suitable dimensions, where it gels after cooling.

It must be remembered that the use of these bridges introduces an additional resistance into the circuit which will change when the bridges become old or when part of the salt diffuses out from the bridges. The resistance should, therefore, be checked at frequent intervals. The error due to the liquid junction potentials is usually so small that it can be neglected. If necessary, it can be determined by adding to the solution an *internal indicator*, *i. e.*, a substance which produces a good polarographic curve with a known half-wave potential.

7. Vessels Used with the Dropping Mercury Electrode

The most important considerations in designing polarographic vessels are: (1) the volume of the solution analyzed; (2) whether or not the analyses are to be carried out in the absence of air; (3) whether an external or internal reference electrode is used; (4) whether or not the work involves routine analyses; (5) how easily the vessel can be cleaned; and (6) what kind of temperature control is available. Of the many designs already in use, only a few typical ones can be mentioned. Modifications of these, meeting individual requirements, will suggest themselves. It

¹⁵⁵ J. P. Baumberger and K. Bardwell, *Ind. Eng. Chem., Anal. Ed.*, **15**, 639 (1943).

should be remembered, however, that when a "pool" reference electrode is used, it must be connected to the circuit by means of a platinum wire.

The simplest type of vessel is an open beaker with a "pool" of mercury on the bottom as reference electrode (see Fig. 25A). For best results, the tip of the capillary should dip at least 2 mm. below the surface of the solution and be at least that far removed from the walls of the vessel and from

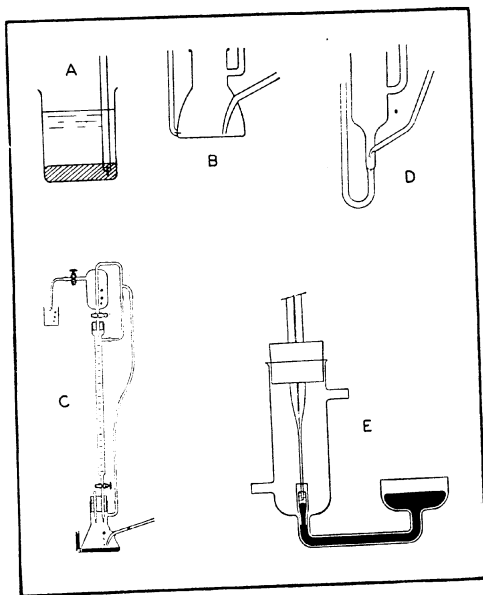


Fig. 25.—Types of vessels used for polarographic analysis.

the bottom layer. For work in air-free solutions, the vessel must be stoppered and an inlet and exit tube for the passage of an indifferent gas must be provided. The most common vessel for such work is the conical vessel *B* (Fig. 25) which is constructed in different sizes, suitable for analyses of 5 ml. of solution or more. When it is necessary to titrate a solution in the absence of atmospheric oxygen, this vessel is combined with a special burette and bubbler,¹⁴³ as shown in figure 25C. The burette is not filled until the titrating solution has been freed from oxygen in the bubbler.

For the analysis of small quantities of solution (0.5–1.0 ml.), vessel *D* of figure 25 has been found satisfactory.¹⁴³ Since the accumulating mercury quickly fills the narrow part of the vessel and makes direct contact

with the capillary, this vessel is suitable only for experiments of short duration. In vessel *E*, this disadvantage is overcome because the wide outside reservoir keeps the mercury level constant. An especially elaborate vessel was designed by Majer¹⁵⁷ in which as little as 0.005 ml. of solution can be freed from atmospheric oxygen and analyzed polarographically. When working with such small quantities of solution, the polarization of the stationary reference electrode may become appreciable,¹⁵⁸ and the material removed by the electrode reaction may significantly alter the solution.

For routine analyses, an external reference electrode is unquestionably preferable to the internal electrodes. The writer has found very convenient a simple arrangement similar to that shown in figure 22 (page 1853). The dropping mercury electrode is clamped firmly in position on a heavy stand and bridge J_2 is attached to it by means of a two-hole rubber stopper in such a way that one end of the bridge is level with the tip of the dropping mercury electrode. The junction vessel, calomel half-cell, and bridge J_1 are connected in suitable positions. A series of solutions, contained in small beakers, can then be analyzed in rapid succession simply by moving each beaker onto a little platform beneath the capillary. Between different samples, the capillary and bridge are washed with distilled water and dried with filter paper, which prevents any measurable contamination of the solutions. When the analyses have to be carried out in the absence of air, vessels of type *B* (Fig. 25) are used and attached to the rubber stopper. A number of solutions can be freed simultaneously from atmospheric oxygen before the analysis. This prevents any delay due to the degassing process.

In other designs, the calomel half-cell and test solution are separated by a porous diaphragm. One such vessel, described by Maassen,¹⁵⁸ is suitable for routine analyses but is apt to disturb and contaminate the calomel half-cell. It is in the shape of a test tube and is provided with a window of porous ceramic material. This vessel is immersed in the supernatant solution of a saturated calomel half-cell, contained in a conical flask, which serves as reference electrode. Lingane and Laitinen¹⁴⁶ recommend an H-shaped vessel in which the calomel half-cell on one side is separated from the test solution on the other side by a sintered glass disk and an agar plug. In the similar "Kalousek" vessel¹⁵⁹ the reference electrode is separated from the test solution by an ungreased stopcock. Both of these vessels are unsuitable for routine analyses and are difficult to clean; furthermore, a new calomel electrode must be prepared for each vessel.

It has already been pointed out (see page 1816) that the temperature a

¹⁵⁷ V. Majer, *Mikrochemie*, **18**, 74 (1935).

¹⁵⁸ G. Maassen, *Angew. Chem.*, **50**, 375 (1937).

¹⁵⁹ R. Strubl, *Collection Czechoslovak Chem. Commun.*, **10**, 475 (1938).

which analyses are carried out should be known with an accuracy of $\pm 0.5^\circ \text{C}$. Room temperature will seldom vary more than that during the time necessary for an experiment, but from day to day it may vary enough to make comparison of results laborious. An automatically controlled thermostat is, therefore, of great convenience for routine work, and most of the designs mentioned above are such that the cell and reference electrode can be immersed in a water bath. The stirrer of the water bath should be so mounted that its vibrations will not jar the dropping mercury electrode, or it should be turned off during the short time necessary for an actual recording of the current-voltage curve. When a range of temperatures is investigated, it is simpler to use as thermostat a large beaker filled with water which can be kept at sufficiently constant temperatures by the addition of either hot or cold water as required.

IV. EXPERIMENTAL TECHNIQUE AND APPLICATIONS

1. General Technique and Applications

The number of organic compounds reducible or oxidizable at a dropping mercury electrode is extremely large, and the application of polarography in the field of organic chemistry seems unlimited. Yet, because of this large number and because most compounds react at potentials that vary with the pH of the solution, polarographic analyses have been unusually difficult. Unless the substance is almost pure, a quantitative and qualitative analysis of a given organic unknown is at present not feasible without preliminary chemical treatment and separation into suitable fractions. Hence a careful combination of electrochemical and general analytical procedures is required to work out the details of a particular analysis. This may become so complicated that other available methods are preferable. In some instances, however, polarography has proved the only workable method of analysis; in others, it has replaced older methods because of its excellent reproducibility, high sensitivity, and other advantageous features. Among these may be mentioned: (1) the rapidity of operation; (2) simultaneous quantitative and qualitative analysis of several components of a solution; (3) the fact that in most cases the solution remains unaltered during the analysis and can be used for other purposes; (4) the possibility of analyzing quantities the size of a drop; and (5) the automatic graph of a recording apparatus which provides a permanent record of the analysis. The simple manual equipment is recommended for preliminary studies and for the novice at polarography, for whom a series of typical experiments has been outlined by Müller.¹⁶⁰ Once a particular

¹⁶⁰ O. H. Müller, *The Polarographic Method of Analysis*. Chemical Education Publishing Co., Easton, Pa., 1941.

procedure has been fully developed, the analyses can be carried out by anyone with some training in chemical analysis. It is well, however, to have expert advice available, since experience has shown that, even in the simplest analyses, irregularities of unforeseen origin are bound to occur.

To be suitable for polarographic analysis, a substance must be electro-reducible or -oxidizable within the range of the electrode. It must be in true solution and it must be stable for the duration of the measurement so that a pure diffusion current can be obtained.

If more than one compound is analyzed at the same time, one must make certain that the starting and end products of either substance do not react with each other and thus alter the curves. A clear-cut example of such interactions is the effect of hydroxyl ions, formed during the reduction of oxygen, on the hydrogen wave of a dilute solution of hydrochloric acid, first observed by Kemula and Michalski.¹⁶¹ Adkins and Cox,¹⁶² found that the wave of acetophenone was markedly lowered by benzalacetone. More extensive studies of Wawzonek and Laitinen,¹⁶³ however, demonstrated that the total diffusion current of acetophenone was unaltered by benzalacetone, but that the shape of the acetophenone wave was affected. Dyes of the eosin group, if present in sufficient concentration, apparently interfere with the reversible oxidation and reduction of numerous oxidation-reduction systems at the dropping mercury electrode. This has been tentatively explained by Wiesner,¹⁶⁴ as caused by the adsorption of eosin.

The potential range over which the dropping mercury electrode can be used depends on the composition of the supporting electrolyte. Müller and Baumberger¹⁶⁵ have shown that, in the absence of ions with which mercury forms complexes or precipitates, potentials as positive as +0.4 v. (*vs. S.C.E.*) can be attained before appreciable oxidation of mercury to mercurous ions takes place. The presence of some anions of organic acids, of halogen, hydroxyl, cyanide, and especially sulfide ions reduces this limit considerably¹⁶⁴ (to as much as -0.6 v., *vs. S.C.E.*). The study of reactions with fairly positive potentials should, therefore, always be carried out in the absence of these ions and an external reference electrode should be used, connected to the cell by a potassium nitrate bridge.¹⁶⁵ In case this is impossible, a careful analysis of the current-voltage curve may still reveal some information if "mixed" potentials¹⁶⁶ are taken into account. For studies of still more positive potentials, other microelectrodes, preferably platinum, must be used.

¹⁶¹ W. Kemula and M. Michalski, *Roczniki Chem.*, **16**, 535 (1936).

¹⁶² H. Adkins and F. W. Cox, *J. Am. Chem. Soc.*, **60**, 1151 (1938).

¹⁶³ S. Wawzonek and H. A. Laitinen, *J. Am. Chem. Soc.*, **63**, 2341 (1941).

¹⁶⁴ K. Wiesner, *Chem. Listy*, **40**, 91 (1946).

¹⁶⁵ O. H. Müller, *J. Chem. Education*, **18**, 320 (1941).

¹⁶⁶ I. M. Kolthoff and C. S. Miller, *J. Am. Chem. Soc.*, **62**, 2171 (1940).

As far as the negative potential limits are concerned, the dropping mercury electrode is unexcelled because of the large hydrogen overvoltage. We have already discussed the most negative potentials attainable with it in the presence of buffers, which can be calculated from equation (39). At $pH > 8$, the alkali and alkaline earth metals limit the potential range (at about -1.8 to -2.2 v.), but Pech¹⁶⁶ found that quaternary amines permitted an extension of this limit to -2.6 v., *vs. S.C.E.* (Laitinen and Wawzonek,¹⁶⁷ using tetrabutyl ammonium iodide, were able to study reductions as negative as -2.8 v., *vs. S.C.E.*). In such media, the interfacial tension of mercury approaches zero as the potential becomes very negative, so that the mercury is dropping very rapidly from the capillary in an almost continuous stream.¹⁶⁸ Naturally, these solutions cannot be buffered, and one must consider possible changes in the pH at the interface brought about by the reaction. Some information about the effect of pH may be had, however, by using different concentrations of the hydroxides of the tetra-alkyl ammonium salts. These hydroxides can be prepared by treating solutions of the purified tetra-alkyl ammonium halides with an excess of moist silver oxide.^{166, 169}

The polarographic method is applicable over a concentration range of 10^{-6} to 10^{-2} molar; for best results, however, a 10^{-4} to 10^{-3} molar solution is desirable. In the latter case, an accuracy of $\pm 1\%$ can be reached by repeating polarograms of the same solution several times¹⁷⁰ and averaging the individual measurements of the diffusion current. This can easily be done if the volume of the solution is 1 ml. or more, because the quantity of material electrolyzed is so small that the body of the solution remains unaltered and subsequent polarograms are identical. When smaller volumes of solution are analyzed, special precautions are necessary, since the quantity of material which is removed by electrolysis and also the end products formed become significant under these conditions. In general, the accuracy and sensitivity of the method are as follows: In practice, 1 ml. of a 10^{-5} *M* solution, or 1 $\mu g.$ of material with a molecular weight of 100, can be analyzed with an accuracy of $\pm 1-3\%$. Under extreme conditions, 0.01 ml. of a 10^{-6} *M* solution, or 0.001 $\mu g.$ of the same material, can be analyzed, but the error is about $\pm 100\%$.

The measurement of the diffusion current, as a rule, presents no difficulties. A preliminary "blank" curve obtained with the supporting electrolyte will indicate the residual current which must be subtracted from

¹⁶⁶ J. Pech, *Collection Czechoslovak Chem. Commun.*, **6**, 126 (1934).

¹⁶⁷ H. A. Laitinen and S. Wawzonek, *J. Am. Chem. Soc.*, **64**, 1765 (1942).

¹⁶⁸ D. Ilkovič, *Collection Czechoslovak Chem. Commun.*, **4**, 480 (1932).

¹⁶⁹ E. S. Peracchio and V. W. Meloche, *J. Am. Chem. Soc.*, **60**, 1770 (1938).

¹⁷⁰ O. H. Müller, *Ind. Eng. Chem., Anal. Ed.*, **14**, 99 (1942).

the test curve to obtain the net diffusion current. This technique is of little help if the unknown produces several waves on the polarogram (Fig. 13, page 1832; Fig. 14, page 1834; Fig. 19, page 1846), or changes the electrocapillary curve of mercury (see p. 1801). In these instances, it is best to draw parallel lines to the beginning and end of the waves, as shown in figure 13. The slope of these lines automatically subtracts the condenser or residual current.

In very dilute solutions, the condenser current complicates the measurement of the diffusion current and is best eliminated or minimized. Ilkovič and Semerano¹⁷¹ have shown that this can be done by means of the circuit drawn in figure 26. The three resistances, R_1 , R_2 , and R_3 can be so chosen that the condenser current (as long as it is linear) is automatically bal-

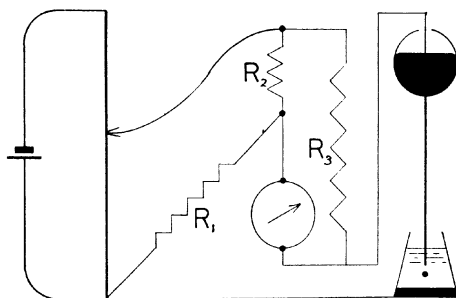


Fig. 26.—Circuit for compensation of the condenser current.¹⁷¹

anced out, or compensated, at any applied voltage by a current of equal magnitude which is sent through the galvanometer in the opposite direction. To prevent any appreciable effect of these additional resistances on the applied voltage and the total circuit resistance, R_1 must be large and R_2 small. The value of resistance R_3 can be calculated¹⁷¹ if the increase in the residual current per volt is known, but it is best determined empirically. The optimum values given by Ilkovič and Semerano are $R_1 = 1000$ ohms, $R_2 = 10$ ohms, and $R_3 = 75,000$ ohms.

Another type of current compensation is required when a polarographic wave is preceded by a much larger diffusion current of a more easily reducible substance (see Fig. 29A, page 1877). Because the first wave is so high, the galvanometer sensitivity must be reduced to such an extent that the second wave becomes too small for accurate measurement. Hohn¹⁷²

¹⁷¹ D. Ilkovič and G. Semerano, *Collection Czechoslovak Chem. Commun.*, **4**, 176 (1932).

¹⁷² H. Hohn, *Z. Elektrochem.*, **43**, 127 (1937).

compensated the first diffusion current by means of an additional electric circuit and battery, so connected that a current of equal magnitude, but in the opposite direction, could be sent through the galvanometer (see Fig. 27). It was then possible to use higher galvanometer sensitivities for the measurement of the second diffusion current.

Since the galvanometer oscillations recorded in such instances are unusually large, because of the high sensitivities used, Lingane and Kerlinger¹⁷³ connected an electrolytic condenser of high capacitance (2000–5000 $\mu\text{f.}$) across the galvanometer shunt (as indicated in Fig. 27). This

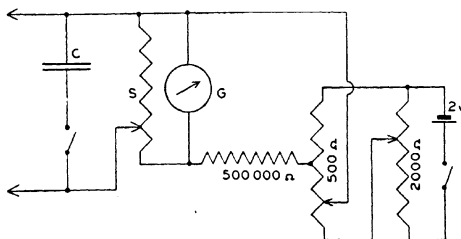


Fig. 27.—Circuit for compensating an interfering diffusion current and for minimizing the galvanometer oscillations. *C*, capacitor of 2000–5000 $\mu\text{f.}$; *G*, galvanometer; and *S*, Ayrton shunt.

reduced the oscillations tremendously without influencing the sensitivity of the galvanometer. The authors showed that the effectiveness of the capacitor is maximal when the net parallel resistance of the galvanometer and shunt is greatest, *i. e.*, when the shunt reduces the galvanometer sensitivity to one-half. They also derived equations for the calculation of the proper capacitor to use in any given case. By introducing a variable resistor (1–300 ohms) in series with the center tap of the shunt¹⁷⁴ and substituting two 1000- $\mu\text{f.}$ electrolytic condensers back to back for the single one of figure 27, a more effective damping is obtained over a wider range of sensitivity and drop-time values.¹⁷⁵ The principle of damping a galvanometer by means of a capacitor was incorporated by Abresch¹⁷⁶ in a portable manual apparatus, called *polarometer*. This instrument is especially suited for polarometric studies (see page 1880) because oscillations of the indicating needle of the microammeter used are practically eliminated.

¹⁷³ J. J. Lingane and H. Kerlinger, *Ind. Eng. Chem., Anal. Ed.*, **12**, 750 (1940).

¹⁷⁴ M. A. Fill and J. T. Stock, *Trans. Faraday Soc.*, **40**, 502 (1944).

¹⁷⁵ G. E. Philbrook and H. M. Grubb, *Anal. Chem.*, **19**, 7 (1947).

¹⁷⁶ K. Abresch, *Chem. Fabrik*, **8**, 380 (1935). See also A. Neuberger, *Z. anal. Chem.*, **116**, 1 (1939).

A most promising technique of *differential polarography* has been developed by Semerano and Riccoboni.¹⁷⁷ In this, two calibrated capillaries, connected to the same mercury reservoir, dip into two solutions, one of which contains the unknown, the other one only the supporting electrolyte. The two anodes which are necessary are connected through suitable resistances: (a) with each other, (b) with the galvanometer, and (c) with the proper end of the slide wire of the polarograph. With this arrangement all currents resulting from the supporting electrolytes balance out; it is therefore not necessary to free the solutions from atmospheric oxygen. Even at voltages at which the alkali metals are deposited, the current flow through the galvanometer is zero if the composition of the two solutions is identical. A practical disadvantage of this method is that, because of small differences in surface tension of the two solutions, the original synchronism of the two dropping mercury electrodes is thrown out of phase so that the current, instead of being steady, will show large undulations around some mean value. This seems to be readily overcome by use of the *streaming mercury electrode* (see page 1882) where the electrode surface is continually refreshed but remains of constant dimensions.¹⁷⁸ It seems to the writer that the knock-off electrode (see page 1861) would likewise overcome these difficulties.

For quantitative work, it is convenient to prepare *calibration curves* in which diffusion current is plotted *vs.* concentration. The concentration of an unknown can then be read directly from the graphs, which must be straight lines passing through zero, if the Ilkovič equation holds. Some authors^{172, 179, 180} measure wave heights by various empirical graphical methods with results differing from the theoretically correct diffusion currents.¹⁸⁴ Hence, calibration curves prepared from such data are often not straight lines and do not pass through zero. The so-called "increment-method,"¹⁸¹ in which the wave height is measured as the difference in current at two applied voltages equidistant from the half-wave potential, is equally arbitrary. Nevertheless, these empirical calibration curves are valuable for practical work, provided that they are prepared by analyzing standard solutions with a composition approximating that of the unknown.

In view of the work of Taylor¹⁸² on the simplest inorganic reductions, the use of *diffusion current constants*¹⁸³ for quantitative work is not likely to be of practical value in organic analyses.

¹⁷⁷ G. Semerano and L. Riccoboni, *Gazz. chim. ital.*, **72**, 297 (1942).

¹⁷⁸ J. Heyrovský, *Chem. Listy*, **40**, 222 (1946).

¹⁷⁹ G. T. Borchardt, V. W. Meloche, and H. Adkins, *J. Am. Chem. Soc.*, **59**, 2171 (1937).

¹⁸⁰ J. K. Wolfe, E. B. Hershberg, and L. F. Fieser, *J. Biol. Chem.*, **136**, 653 (1940).

¹⁸¹ H. G. Petering and F. Daniels, *J. Am. Chem. Soc.*, **60**, 2796 (1938). R. H. Müller and J. F. Petras, *ibid.*, **60**, 2990 (1938).

¹⁸² J. K. Taylor, *Anal. Chem.*, **19**, 368 (1947).

¹⁸³ J. J. Lingane, *Ind. Eng. Chem., Anal. Ed.*, **15**, 583 (1943).

If the unknown is more concentrated than $10^{-3} M$, it is usually possible to work with the solutions open to air, but the current of the oxygen curve must be subtracted from the "test" curve. In more dilute solutions, the atmospheric oxygen must be removed by chemical means (with sodium sulfite in neutral or alkaline solution) or by bubbling an inert gas (hydrogen, nitrogen) through the solution. The latter method is preferable because it does not alter the composition of the solution except in the presence of volatile constituents. In such cases, complications are avoided by passing the gas through a wash bottle containing some of the solution to be analyzed, before bubbling it through the test solution. In all other instances, the gas should be saturated with water vapor before passage through the electrolysis vessel, to prevent evaporation of water from the test solution. The gases used should be tested polarographically for their oxygen content; traces of oxygen which may be present can be removed by passing the gas through heated copper turnings or through oxygen-absorbing solutions. Of course, the flow of gas through the test solution must be stopped during the recording of a polarogram because the drops of mercury should never be forced off by motion in the solution but should fall under their own weight.

In addition to the comparison with calibration curves, two other methods are available for quantitative analysis. The *method of standard addition*¹⁸⁴ is particularly advantageous in isolated determinations. In this method, a polarogram is first prepared with m ml. of a solution of unknown concentration, X . Then n ml. of a standard solution of the same reactant (of concentration C) is added, and a second polarogram is recorded. The observed diffusion currents are, then, according to the Ilković equation:

$$I_1 = kX \qquad I_2 = k(mX + nC)/(m + n)$$

from which can readily be deduced that:

$$X = C \frac{nI_1}{I_2(m + n) - mI_1} \qquad (40)$$

The accuracy of this method depends on the precision with which the two volumes of solution and the corresponding diffusion currents are measured.

The *method of step quotients* was suggested by Forche¹⁸⁵ to simplify polarographic work with different capillaries and in solutions of different viscosity. Working with mixtures of cadmium and lead, this author found that the *ratio* of the wave heights of these two ions in any given solution was independent of the capillary used and of the viscosity of the medium.

¹⁸⁴ H. Hohn, *Chemische Analysen mit dem Polarographem*. Springer, Berlin, 1937.

¹⁸⁵ H. E. Forche, *Mikrochemie*, 25, 217 (1938).

On the basis of the Ilkovič equation, it could have been predicted that:

$$\frac{I_{dp}}{I_{du}} = \frac{n_p D_p^{1/2}}{n_u D_u^{1/2}} \frac{C_p}{C_u} \quad (41)$$

where the subscripts p refer to a chosen "pilot ion" and the subscripts u to the unknown. The quantity $n_p D_p^{1/2} / n_u D_u^{1/2}$ may be called the "step quotient." Apparently it does not vary appreciably with the viscosity of the solution, and it will probably be influenced but little by temperature (except when H^+ or OH^- are involved). Precise information on a great number of pairs of reactants is needed to justify these predictions.

Once the step quotient of a given unknown has been determined with carefully prepared solutions of known concentrations, any other concentration of this unknown can be found as follows: A standard solution of the "pilot ion" is prepared and a known volume of it is added to a known volume of the test solution. The solution is then polarographed and C_u is calculated from the ratio of the observed diffusion currents, the known concentration C_p , and the step quotient. For highest accuracy, the wave height of the two reactants should be about equal. It is essential, of course, that the unknown solution originally contain none of the material which is used as the pilot ion. If the step quotient is really independent of the medium and temperature, this method offers great possibilities, since it requires only a single standard solution and is especially adaptable to series analyses in which a number of different capillaries are used in rotation. It is doubtful, however, if this method can be applied to the analysis of compounds producing several waves of unequal ratios on the polarograms.

Besides this quantitative application, the pilot-ion technique has been used for some time for potential measurements in which the half-wave potential of the pilot ion, or indicator, was taken as the standard of reference (see Heyrovský and Müller,¹²⁷ and Novák¹²⁷). As mentioned previously, this is only permissible when the potential of the large electrode remains constant.

Qualitative as well as quantitative analysis, in general, becomes increasingly difficult as the number of reducible or oxidizable substances in a given solution increases, because the waves often overlap or come so close together that their resolution is impossible. To demonstrate the need for separation and for a favorable relation of the heights of individual waves, figures 28 and 29 were prepared.¹⁶⁴ The curves shown are theoretical current-potential curves of two reversible reductions at 30° C., calculated for a two-electron change per molecule. Since these are current-potential curves, they are independent of the actual scale of the current axis, *i. e.*, the curves should look the same whether the sum of the two waves is 10 or 1000 microamperes. In figure 28, the ratio of the two waves is unity; in

figure 29, the more positive wave is ten times as high as the more negative wave in the upper series A, while the opposite is true in the lower series B.

The half-wave potentials of the waves of figure 28 are indicated by arrows. They are separated by 0, 30, 60, 90, 120, and 180 mv., respectively. It may be seen that a difference of at least 90 mv. between the half-wave potentials is necessary to produce measurable waves. If the difference is only 30 mv., the waves overlap to such an extent that a determination of wave heights becomes impossible. A separation of 60 mv. in this case is

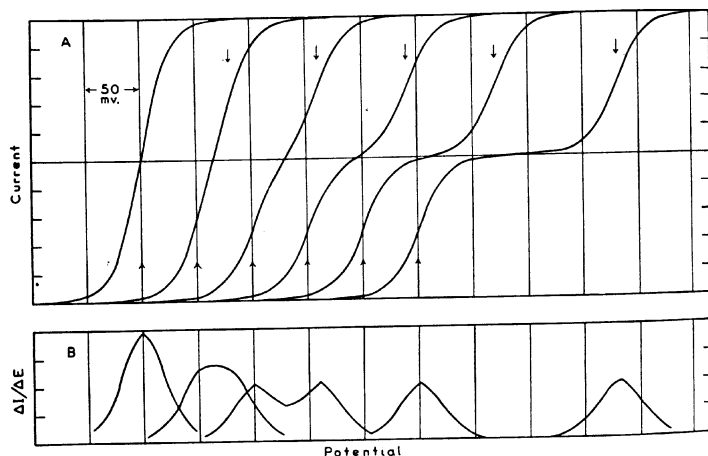


Fig. 28.—(A) Theoretical current-potential curves of two waves of equal height with half-wave potentials (indicated by arrows) separated by 0, 30, 60, 90, 120, and 180 mv. (B) $\Delta I / \Delta E$ of the above curves plotted against the potential.¹⁸⁴

just enough to see that there are two inflections, but no accurate measurements can be made. If the change of current per unit change in potential $\Delta I / \Delta E$ is plotted against the potential, as in part B of figure 28, a sharp peak will be obtained at the half-wave potential of each wave. In a double-wave, two such peaks will be observed if the waves are separated sufficiently. These increment ratios are often used in potentiometry, but have been adapted to polarography only recently. Using a very simple circuit, Heyrovský¹⁸⁶ was able to plot such "derivative curves" automatically with the polarograph.

Curves with similar separations of the half-wave potentials, but with a less favorable ratio of the two waves, are shown in figure 29. It may be

¹⁸⁶ J. Heyrovský, *Analyst*, 72, 229 (1947); *Chem. Listy*, 43, 149 (1949).

observed that, even at a separation of 90 mv., the measurement of the waves is somewhat difficult. The small waves can be measured with greater accuracy if they are magnified by increasing the galvanometer sensitivity; in the upper series A, the first diffusion current must be compensated (see page 1872) to make this possible.

These same figures (28 and 29) can be used for a consideration of reactions involving one electron per molecule, by doubling the scale of the abscissa. A minimum of 180 mv. is then found necessary for a satisfactory

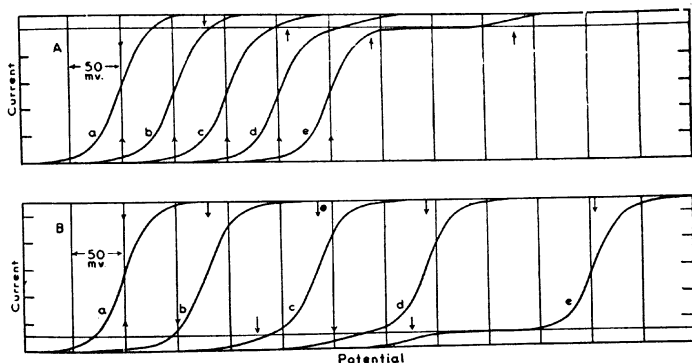


Fig. 29.—Theoretical current-potential curves of two waves with half-wave potentials (indicated by arrows) separated by 0, 30, 60, 90, and 180 mv.¹⁶⁴ The heights of these waves are in ratios of (A) 10:1 and (B) 1:10.

separation of the half-wave potentials, leading to the general conclusion that, for measurements of simultaneous reductions, the midpoints of waves must be separated by at least $RT/nF \ln 1000$.

The *resistance of the circuit*, which must be known for all *IR* corrections, can be determined in several ways. Undoubtedly, the conventional measurement by means of a Wheatstone bridge is the most accurate. With this method, one finds a minimum resistance just before the drop falls off, and Ilkovič¹⁶⁸ has shown that this value must be multiplied by $4/3$ to represent the mean resistance necessary in our calculations. The latter can also be obtained directly from the polarographic curves. For instance, (a) the slope of a steep maximum,¹⁶⁸ as in the case of the oxygen reduction (Fig. 4, page 1799), (b) the slope of a line combining the anodic and cathodic half-wave potentials of a reversible system, polarographed both in the oxidized and the reduced forms,⁷³ and (c) the slope of a line passing through the half-wave potentials of a given substance reduced at different

concentrations under otherwise identical conditions (Fig. 17, page 1843), are all equal to the reciprocal of the resistance of the circuit, in accordance with Ohm's law. In still another method,¹⁶⁰ different voltages are applied to the cell from a *B* battery and the corresponding current is noted on a milliammeter. By means of Ohm's law, resistances are calculated which approach a limit as the applied voltage increases past 9 or 10 volts. Even better results may be obtained if the voltage is plotted against the observed current; it will be found that, after a certain minimum voltage is exceeded, the current increases almost linearly with a slope equal to the reciprocal of the cell resistance, as above.

Since it is not necessary to know the circuit resistance more accurately than to a few hundred ohms, the resistance of the capillary itself (about 50 ohms) and that of the potentiometer and lead wires can usually be neglected. However, the resistance of the galvanometer and shunt may become appreciable, depending on the type used and on the setting of the shunt (see page 1855).

2. Specific Techniques and Applications

The available data suggest that, in general, all those organic compounds are reducible at the dropping mercury electrode which are reducible by chemicals (including dissolving metals), as contrasted with catalytic reductions.¹⁸⁷ Thus, for instance, simple purely aliphatic olefins are not reducible at the dropping mercury electrode, while the presence of an aryl, carbonyl, or ethylene group, conjugated with the unsaturation, makes this reduction possible. With the extension of the potential range of the dropping mercury electrode to potentials as negative as -2.9 v. (*vs. S.C.E.*), measurable polarographic waves have been obtained even with a number of phenyl-substituted olefins and acetylenes¹⁶⁷ and aromatic polynuclear hydrocarbons,¹⁸⁸ although their half-wave potentials are all more negative than -2.0 v.

In general, organically bound halogen is reducible. Apparently this reduction is independent of the *pH* of the solution, a fact which can be used to recognize the type of reaction in complicated cases.¹⁸⁹

Sometimes it may be possible that an organic substance which is not reducible at the dropping mercury electrode can be altered in such a way that it becomes electroactive and, consequently, determinable. Perhaps the best example of a combination of organic chemistry and polarography is the work of Fieser and collaborators^{190, 191} who, by a treatment of the

¹⁸⁷ K. N. Campbell and B. K. Campbell, *Chem. Revs.*, **31**, 77 (1942).

¹⁸⁸ S. Wawzonek and H. A. Laitinen, *J. Am. Chem. Soc.*, **64**, 2365 (1942).

¹⁸⁹ R. Pasternak and H. v. Halban, *Helv. Chim. Acta*, **29**, 190 (1946).

¹⁹⁰ E. B. Hershberg, J. K. Wolfe, and L. F. Fieser, *J. Biol. Chem.*, **140**, 215 (1941).
J. K. Wolfe, L. F. Fieser, and H. B. Friedgood, *J. Am. Chem. Soc.*, **63**, 582 (1941).

nonelectroactive 17-keto-steroids, androsterone and isoandrosterone, and of dehydroisoandrosterone with Girard reagent T (trimethyl acethydrazide ammonium chloride), obtained reducible substances.

Applications in Problems of Theoretical Interest.—In addition to such problems as tautomerism, polymerization, the effect of pH, catalysis, and stepwise reductions, for which examples have already been given in the theoretical part, numerous studies have been carried out on the effect of substitution in the organic molecule on the reduction potential. In potentiometry, similar investigations are limited to reversible systems and to a narrow range of potentials but, in polarography, irreversible systems can be studied equally well and in the overvoltage range. Some contributions have also been made by polarography in the determination of the structure of organic compounds.

Another application for which the polarographic method is well suited is the study of reaction kinetics or photochemical reactions, when the reactions are fairly slow. In these studies, continuous analyses of the reaction mixtures may be carried out at constant applied voltages and recorded as current-time curves (see polarometric analysis, page 1880), or complete polarograms may be recorded of the reaction mixture or of samples removed from it at known intervals of time.

Polarography as an Aid in Organic Synthesis.—In organic synthesis, polarography may be used to determine the purity of the product and the effectiveness of various stages in its synthesis. In such an application, the analyses may often be carried out in a very empirical way, because the nature of the side products need not be known. In the field of electro-organic synthesis, the polarographic method may indicate the most efficient procedure for the execution of a given reaction.^{191,192}

Reactions Involving Oxidation of Mercury.—If the dropping electrode is made sufficiently positive, it changes from an indicator to an active electrode and mercury goes into solution. Revenda¹⁹³ found that such anodic currents reached limiting values depending on the concentration of inorganic ions (*e. g.*, Cl⁻, CN⁻, S²⁻) which precipitate or form complexes with mercurous ions. Similar curves should be expected in the case of organic compounds which form complexes with mercurous ions, and Kolthoff and Barnum¹⁹⁴ found cysteine to behave in this manner.

Analyses Involving the Suppression of Maxima.—Besides catalytic reactions, the most sensitive type of analysis is that in which the adsorption of organic compounds at the dropping mercury electrode is used for quan-

¹⁹¹ H. Siebert, *Z. Elektrochem.*, **44**, 768 (1938); **45**, 228 (1939).

¹⁹² J. J. Lingane, C. G. Swain, and M. Fields, *J. Am. Chem. Soc.*, **65**, 1348 (1943).

¹⁹³ J. Revenda, *Collection Czechoslovak Chem. Commun.*, **6**, 453 (1934).

¹⁹⁴ I. M. Kolthoff and C. Barnum, *J. Am. Chem. Soc.*, **62**, 3061 (1940).

titative determinations. While the theory of maxima and even the quantitative aspects of maximum suppression are still uncertain, several empirical applications of this technique have been possible.¹⁹⁵

Analysis of Volatile Substances.—When volatile substances are analyzed polarographically, special precautions must be taken (see page 1874) to avoid loss of material during the degassing of the solution. Gniubkin, Dobrinskaja, and Neumann¹⁹⁶ found that, under certain conditions, this loss in material by volatilization can be used for analytical purposes.

Polarometric Determinations.—Anyone preparing polarograms with the simple manual equipment must have realized that, whenever a plateau is reached at the end of a polarographic wave, a constant applied voltage can

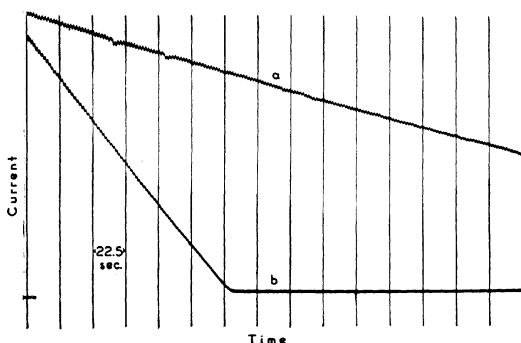


Fig. 30.—Polarographic record of current-time curves representing rates of O_2 consumption of yeast cells: (a) in absence of substrate; and (b) in presence of glucose.¹⁶⁴

be used for the determination of the substance. At this fixed voltage, the diffusion current should be a linear function of the concentration. In making this simplified assumption, however, one must be sure that (a) the diffusion current is corrected for the residual current and any current which might be caused by the preceding discharge of another ion, and (b) no significant error is produced by small variations in the applied voltage or by changes in the electrode potential due to different IR corrections with changes in current.¹⁶⁴ One should therefore select for such measurements an applied voltage at which a level plateau in the polarographic curve indicates that small changes in the applied voltage produce no measurable

¹⁹⁵ R. Procházka, *Chimie & industrie, Special Number*, pp. 281–283 (June, 1933). B. Gosman and J. Heyrovský, *Trans. Electrochem. Soc.*, **59**, 249 (1931).

¹⁹⁶ V. I. Gniubkin, A. A. Dobrinskaja, and M. B. Neumann, *Acta Physicochim. U. R. S. S.*, **11**, 701 (1939).

change in the diffusion current. Numerous applications have been published of such analyses; they have so far dealt largely with inorganic substances, but are equally suitable for organic compounds. These applications fall into two classes, polarometric studies of reaction kinetics and polarometric titrations.

In *polarometric studies of reaction kinetics*, a suitable fixed voltage is applied to the cell and the change in current is observed over a period of time. By means of the polarograph, a record can be prepared automatically. This method has been most widely used in the study of oxygen consumption by biological materials. An example is shown in figure 30, where the rate of oxygen consumption of yeast cells is given by the slope of the current-time curves.¹⁶⁴ (The applied voltage was constant at -0.5 v. vs. S.C.E.) Note that the rate increases in the presence of substrate, but is independent of the oxygen concentration. Investigations of this sort have already given much interesting information,¹⁹⁷⁻²⁰⁰ especially in combination with colorimetric and potentiometric data which could be simultaneously recorded on the polarograms.¹⁹⁷

In *polarometric titrations*,²⁰¹ the current at a constant applied voltage is observed while known quantities of a titrating agent are added to the solution. From a graph of the data, the end point of the titration can be determined. This type of analysis is suitable for precipitations, neutralizations, or oxidation-reduction titrations. To account for the change in volume of the solution during the titrations, the observed currents should be multiplied by the factor, $(V + X)/V$, where V is the starting volume of the solution and X is the volume of titrating agent added.²⁰¹ Thus, usually straight lines are obtained which permit extrapolations to the end points.

Figure 31 shows four of the most common types of curves obtained in polarometric titrations. In *A*, the electroactive substance is removed from the solution by precipitation with an inactive substance (e. g., lead titrated with sulfate ion²⁰¹). In *B*, to an inactive substance an electroactive precipitating agent is added (e. g., sulfate titrated with barium ion²⁰¹). In *C* both the component of the solution and the precipitant are reducible at the given applied voltage (e. g., lead titrated with dichromate ion²⁰²). Finally, in *D*, the titrating agent produces a linear decrease in current until negative values are reached. This type of curve may be obtained by an oxidative-reductive titration (e. g., titanium titrated with ferric ion²⁰³) or by precipi-

¹⁹⁷ J. P. Baumberger, *Cold Spring Harbor Symposia Quant. Biol.*, **7**, 195 (1939).

¹⁹⁸ R. J. Winzler, *J. Cellular Comp. Physiol.*, **17**, 263 (1941).

¹⁹⁹ E. G. S. Baker and J. P. Baumberger, *J. Cellular Comp. Physiol.*, **17**, 285 (1941).

²⁰⁰ C. O. Warren, *J. Cellular Comp. Physiol.*, **19**, 193 (1942).

²⁰¹ V. Majer, *Z. Elektrochem.*, **42**, 120, 123 (1936).

²⁰² A. Neuberger, *Z. anal. Chem.*, **116**, 1 (1939).

²⁰³ R. Strubl, *Collection Czechoslovak Chem. Commun.*, **10**, 475 (1938).

tation of a polarographically determinable anion with a suitable cation (e. g., chloride titrated with silver ion¹⁶⁴). The end point in this case is reached when the current becomes zero (corrected for any residual current), i. e., where the straight titration curve intersects the galvanometer zero line. These polarometric titrations are fast and accurate, but have limitations similar to conductivity measurements.

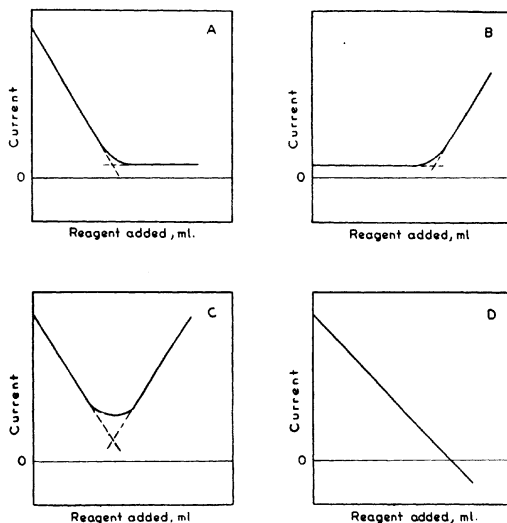


Fig. 31.—Types of polarometric titration curves (see text).¹⁶⁴

Numerous polarometric titrations have been developed for inorganic reactions, mainly by Kolthoff *et al.* (who prefer to call these *amperometric* titrations). An example of a polarometric titration of an organic compound is the titration of α -tocopherol with auric chloride,²⁰⁴ which belongs to type A (Fig. 31) mentioned above. An entirely organic polarometric titration was recently carried out by Cohn and Kolthoff.²⁰⁵

Polarography with Microelectrodes Other Than Dropping Mercury.—Complications arising as a consequence of: (a) the growth of the surface of the dropping mercury electrode, and (b) irregularities in dropping because of sudden changes in surface tension can be overcome by a *streaming mer-*

²⁰⁴ L. I. Smith, I. M. Kolthoff, and L. J. Spillane, *J. Am. Chem. Soc.*, **64**, 646 (1942).

²⁰⁵ G. Cohn and I. M. Kolthoff, *J. Biol. Chem.*, **148**, 711 (1943).

cury electrode.²⁰⁶ It consists of a fine stream of mercury which is forced through a capillary from beneath the solution to its surface; the length of the stream (4–8 mm.) is regulated by the position of the capillary below the surface of the solution. This streaming mercury electrode is especially well suited for studies with a cathode-ray tube, because one can easily obtain with it oscillograms with standing waves which are suitable for direct measurement. It furthermore offers some definite advantages over the dropping mercury electrode in differential polarography (see page 1873).²⁰⁶

The limitation of the dropping mercury electrode in the positive potential range and the fact that many organic reactions are catalyzed by different electrode materials might render other microelectrodes particularly important in organic chemistry. Such electrodes have been studied for many years but they saw no practical use until knowledge gained with the dropping mercury electrode made their behavior more comprehensible.^{207, 208}

In a special study of different kinds of microelectrodes, Kolthoff and Laitinen²⁰⁹ compared wire and spherical electrodes and various flat electrodes which were either mounted at the end of glass tubes or recessed in glass tubes and so placed that downward, upward, and horizontal convection and diffusion could be studied. The results obtained with the recessed electrode with upward linear diffusion agreed best with theory, but from a practical standpoint the wire electrode was chosen by the authors as most satisfactory for general use. If the cell is protected from all thermal and mechanical disturbances and one waits for 2 minutes or longer after applying a given voltage, a "steady-state" diffusion current can be obtained; naturally the preparation of current-voltage curves by this means becomes very tedious. To eliminate this disadvantage, Laitinen and Kolthoff²¹⁰ mounted a small platinum wire on the side of a shaft which was rotated at 1800 r. p. m. With this rotating microelectrode the diffusion current and therefore the sensitivity of the method is tremendously increased.

A stationary platinum microelectrode sealed into the most narrow part of a constricted glass tube, past which an electrolyte is made to flow, was employed by Müller²¹¹ for the automatic polarographic recording of current-voltage curves. The results are reproducible as long as the following conditions are kept constant: (a) rate of flow of solution past the electrode,

²⁰⁶ J. Heyrovský and J. Forejt, *Z. physik. Chem.*, **193**, 77 (1943); J. Heyrovský, *Chem. Listy*, **40**, 222 (1946); J. Heyrovský, F. Šorm, and J. Forejt, *Collection Czechoslovak Chem. Commun.*, **12**, 11 (1947).

²⁰⁷ L. R. Blinks and R. K. Skow, *Proc. Natl. Acad. Sci.*, **24**, 420 (1938).

²⁰⁸ P. W. Davies and F. Brink, *Am. J. Physiol.*, **133**, P257 (1941); *Rev. Sci. Instruments*, **13**, 524 (1942).

²⁰⁹ H. A. Laitinen and I. M. Kolthoff, *J. Am. Chem. Soc.*, **61**, 3344 (1939).

²¹⁰ H. A. Laitinen and I. M. Kolthoff, *J. Phys. Chem.*, **45**, 1061, 1079 (1941).

²¹¹ O. H. Müller, *J. Am. Chem. Soc.*, **69**, 2992 (1947).

(b) rate and direction of change of the applied voltage, (c) temperature, (d) pretreatment of the electrode, and (e) kind and concentration of the supporting electrolyte. Under proper conditions the limiting currents obtained with this "by-pass" electrode are a linear function of: (1) the concentration of the reacting substance, and (2) the logarithm of the rate of flow, so that it can be used to measure either of these quantities or a combination of the two, the *availability of material* per unit time.

In order to overcome polarization difficulties with solid microelectrodes Miller²¹² has used two of them alternately in conjunction with a large non-polarizable electrode; while one of them is active, the other is short-circuited to the large electrode and thus "discharged." A similar discharge technique of but a single microelectrode by means of a commutator has been used by Olson, Brackett, and Crickard.²¹³

Attention must be called to the fact that, whenever organic oxidation-reduction systems were studied with these solid microelectrodes, certain irreversible phenomena became apparent which were absent when only inorganic ions were involved. Müller²¹¹ suggested that these may be caused by slowness in the dissociation of the buffers and components of the system, and that such electrodes may serve as tools in the investigation of reaction rates which are too fast to be measured with conventional techniques.

²¹² S. D. Miller, *Trudy Vsesoyuz. Konferentsii Anal. Khim.*, **2**, 551 (1943).

²¹³ R. A. Olson, F. S. Brackett, and R. G. Crickard, *J. Gen. Physiol.*, **32**, 681 (1949).

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von Stackelberg, M., "Die wissenschaftlichen Grundlagen der Polarographie." *Z. Elektrochem.*, **45**, 466 (1930).

CHAPTER XXIX

Determination of MAGNETIC SUSCEPTIBILITY

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I. INTRODUCTION

Out of the vast volume of material, experimental and theoretical, in the field of magnetism, there is only a small part which at the present time is

useful in work on the problems of organic chemistry.¹⁻⁶ Among the numerous methods available for the measurement of the properties which are important to organic chemistry, only those few will be selected which appear to be most convenient for the purpose. Very likely many other theoretical and experimental procedures will be resorted to in the near future.

Since this chapter is principally concerned with methodology, it cannot be devoted to an extended presentation of the theory of magnetism. On the other hand, the interpretation of experimental results is based upon theory. Thus the manner and extent of discussing the theoretical background involve a compromise with respect to the completeness and rigidity of the theoretical discussions.

II. DIAMAGNETISM AND PARAMAGNETISM

All matter is either diamagnetic, paramagnetic, or ferromagnetic. Ferromagnetism plays no role in organic chemistry and requires consideration here only insofar as contamination, even with slight traces of ferromagnetic impurities, may cause considerable errors in the magnetic measurements of solid organic materials. Every molecular species is diamagnetic. Paramagnetism, if present, is superimposed on diamagnetism. For any single molecular species, the paramagnetic effect, if present at all, is much stronger than the diamagnetic one.

The response of a particle to a magnetic field is twofold, an orientation with respect to its axes, and a displacement. An orientation takes place whenever the magnetic properties of the particle are different along different axes, either when the particle is intrinsically magnetically anisotropic, as in many crystals, or when the particle, although intrinsically isotropic, is not a sphere. This latter effect, due to asymmetry in external shape alone, is exhibited only in an inhomogeneous field, and even then is small. The axis of greatest paramagnetism, or of smallest diamagnetism, orients itself along the direction of the field lines. Much more important for most methods to be described here is the displacement. It occurs only when the particle is exposed to an inhomogeneous field. A paramagnetic particle moves in the direction of increasing field strength, and a diamagnetic one, in the direction of decreasing field strength.

¹ E. C. Stoner, *Magnetism and Matter*. Methuen, London, 1934.

² J. H. Van Vleck, *Electric and Magnetic Susceptibilities*. Oxford Univ. Press, New York, 1932.

³ S. S. Bhatnagar and K. N. Mathur, *Physical Principles and Applications of Magnetochemistry*. Macmillan, London, 1935.

⁴ W. Klemm, *Magnetochemie*. Akadem. Verlagsgesellschaft, Leipzig, 1936.

⁵ L. Pauling, *The Nature of the Chemical Bond*. Cornell Univ. Press, Ithaca, 1939.

⁶ P. W. Selwood, *Magnetochemistry*. Interscience, New York, 1943.

The methods to be described here are concerned essentially with the measurement of the force of such a displacement. That magnitude which is directly accessible to measurement and correlated with the force of displacement is the *magnetic susceptibility*. It is positive for a paramagnetic, and negative for a diamagnetic substance. From the susceptibility there is derived, by theoretical reasoning, the magnetic dipole moment. A paramagnetic substance has a permanent magnetic dipole moment. A diamagnetic substance has no magnetic dipole moment in the absence of a magnetic field, but acquires an induced magnetic moment when exposed to the field. The induced magnetic moment for any molecular species is always negative, and relatively weak. A permanent magnetic dipole moment, if it exists at all, is always positive and comparatively strong. In this respect there is no analogy for the general behavior of magnetic and electric dipole moments. Paramagnetic susceptibility depends on temperature; diamagnetism does not. In a mixture of two or more molecular species, susceptibility is additively composed of the susceptibilities of the components (Wiedemann's law of additivity of susceptibilities). It should be kept in mind that this additive rule holds for susceptibilities but not for dipole moments.

A knowledge of the magnetic dipole moment of a molecular species can be utilized with respect to the elucidation of the structure of the molecule and of the electronic configuration of the atoms. Paramagnetism occurs only in the following two classes of molecular species: those containing atoms of the transitional group of the periodic system (such as iron, nickel, cobalt); and those containing an uneven number of electrons, the "odd molecules," or "free radicals." In a few cases, a molecule may contain an even number of electrons, two of which are not paired. These molecules are also paramagnetic. The best known example is oxygen. However, some organic molecules of this type have recently been discovered. They are termed biradicals.⁷

In organic chemistry, the main concern with magnetism lies in the study of the free radicals. Provided no heavy metal atom of the transitional group is present, paramagnetism is an unambiguous criterion for free radicals, the biradicals being included in this group, and can be utilized for their identification and even for their quantitative determination in mixtures with other molecules which are not free radicals.

All organic molecules with an even number of electrons, all of which are paired, or the "valence-saturated compounds," are diamagnetic. At

⁷ R. Kuhn, H. Katz, and W. Franke, *Naturwissenschaften*, **22**, 808 (1934). E. Müller and I. Müller-Radloff, *Ann.*, **521**, 81 (1935). As regards the existence of a biradical as an excited state of a diamagnetic molecule, see G. N. Lewis and M. Kasha, *J. Am. Chem. Soc.*, **66**, 2100 (1944).

first approximation, the diamagnetic susceptibility of a molecule is additively composed of the susceptibilities of its atoms. The specific contribution of each atomic species has been evaluated empirically by Pascal.³ If this additivity rule were strictly true (and it is not), diamagnetic properties would be of no avail at all for structural analysis. According to special properties, such as double bonds and other structural features, slight "structural corrections" to the additivity rule must be applied in order to obtain values in agreement with the experimental measurements; vice versa, the deviations of the observed values from those obtained by the additivity rule may be utilized for structural analysis.

The necessity of taking into consideration the diamagnetic properties even for paramagnetic molecular species is due to the fact that all magnetic measurements reveal only the total, or resultant, magnetic susceptibility which, for free radicals, is composed of the paramagnetic and diamagnetic susceptibilities. The diamagnetic one is always weak and of opposite sign to the paramagnetic one. It is only the paramagnetic contribution which is important in the study of free radicals. In order to obtain the purely paramagnetic effect, it is necessary to subtract the diamagnetic one. This correction may be of very different magnitude for different systems. When a paramagnetic pure chemical compound, solid or liquid, is being measured, the diamagnetic effect is always small. On the other hand, if a solution of a paramagnetic molecular species in a diamagnetic solvent such as water is measured, the paramagnetic effect of the solute may be manifested sometimes just by lowering slightly the diamagnetic effect of the solvent.

From these remarks it follows that it will be necessary to discuss: (1) the experimental methods for the measurement of the total, or resultant, susceptibility; (2) for paramagnetic substances, the methods for finding the corrected paramagnetic susceptibility, freed from all diamagnetic effects, the calculation therefrom of the permanent magnetic dipole moment, and its interpretation in terms of structural chemistry; and (3) for diamagnetic molecules, the interpretation of susceptibility in terms of structural chemistry.

III. MEASUREMENT AND CALCULATION OF MAGNETIC SUSCEPTIBILITY

1. General Considerations

Magnetic susceptibility will be defined here in terms of the experimental operations necessary to arrive at the numerical value of the susceptibility of a substance.

³ P. Pascal, *Ann. chim. phys.*, numerous papers from 1910 to 1925.

On exciting an electromagnet, a magnetic field will arise in the space between the poles (*N* and *S*, Fig. 1). For the pole pieces considered here, with parallel, plane circular surfaces of equal area, the field can be symbolized in terms of Faraday's force lines, as shown in the figure. In the neighborhood of the *X* axis the lines are both parallel and equidistant, symbolizing the homogeneity of this part of the field. Farther up and down along the *Z* axis the lines are curved and the distance between neighboring lines increases with the distance from the *X* axis, indicating that the field is inhomogeneous, its strength gradually becoming weaker and finally vanishing. Even for a strong electromagnet with a pole gap of, for instance, 1 cm., and a diameter at the pole surfaces of 3 cm., the field strength, which is highest in the homogeneous region, diminishes rapidly along the *Z* axis and is negligibly small at, say, 15 cm. or so, according to circumstances, above and below the pole pieces. A particle, either paramagnetic or diamagnetic, is not displaced when exposed to the homogeneous part of the field. When placed at some point in the inhomogeneous field, it moves toward increasing field strength if it is paramagnetic, and toward decreasing field strength if it is diamagnetic.

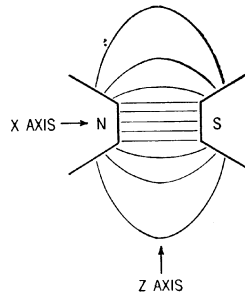


Fig. 1.—Field lines.

A piece of iron, however, when placed even in the homogeneous field, will move along the *X* axis and be attracted by one pole. This phenomenon is accounted for by the fact that a substance of such high magnetizability as iron displaces the lines of force and distorts the field sufficiently to establish a component of the force along the *X* axis. Less magnetizable substances, which do not distort the applied field to any appreciable extent, when placed in the inhomogeneous part of the field, will move in the direction, not of the *X* axis, but of the *Z* axis. The pull is greatest where the inhomogeneity of the field is greatest. The inhomogeneity of the field is measured in terms of $\partial H/\partial Z$, where *H* is the field strength measured in "gauss." (Sometimes the term "oersted" is used synonymously.) For any material which is not ferromagnetic, the pull at any point of the field is proportional both to the field strength, *H*, at this point, and its inhomogeneity at this point, $\partial H/\partial Z$, and so the pull is proportional to:

$$H \cdot \partial H / \partial Z = \frac{1}{2} \cdot \partial H^2 / \partial Z \quad (1)$$

as can be inferred from the following consideration. The force on a particle of moment μ , exerted along the *Z* axis in a magnetic field of strength *H*,

depends on the inhomogeneity of the field along this axis, dH/dZ . On the other hand, a particle of susceptibility, κ , in a magnetic field, H , acquires the moment κH ; thus the pull, F , exerted on a particle of susceptibility, κ , is:

$$F = \kappa H \cdot \partial H / \partial Z \quad (2)$$

If the test object is a long, homogeneous cylinder, extending from some region within the homogeneous part of the field upward to a region of vanishingly small field strength, the total pull exerted on the cylinder will be the integral of all differential pulls on its horizontal differential layers. This pull is independent of the length of the cylinder provided the cylinder reaches from the region of the homogeneous field to a region of vanishingly small field strength. The total pull on the cylinder is proportional to the square of the field strength of the homogeneous part of the field. This pull can be measured by compensating it, with the aid of a balance, by a counterweight. This apparent increase, for a paramagnetic substance, or decrease for a diamagnetic one, in weight due to the magnetic field is proportional to the magnetic susceptibility of the cylinder. The proportionality factor with which the apparent weight increase, measured in terms of milligrams, must be multiplied in order to agree with the centimeter-gram-second electromagnetic units (c.g.s.u.), according to which susceptibility is defined, is obtained by a calibration. This calibration can be carried out by measuring, under the same conditions of the instrument, another object of the same shape, of known susceptibility, which has been previously measured by methods suitable for the measurement of absolute instead of relative susceptibilities.

Thus the magnetic pull has been shown to be proportional to the square of the magnetic field in its homogeneous region. This holds for the assumption that the field strength at the upper end of the cylinder is negligible. More generally speaking, when the field strength at the lower and the upper ends of the cylinder are H_1 and H_2 , respectively, the pull is proportional to $(H_1^2 - H_2^2)$.

Since the substance to be measured is usually filled into a cylindrical glass vessel, the magnetic properties of the vessel must be considered too. This can be easily circumvented by measuring successively in the same glass container two different substances of known susceptibilities. The difference between the two pulls is proportional to the difference between the susceptibilities of the two substances, and the proportionality factor for converting the apparent change in weight into rational units of susceptibility is thereby obtained. It must be borne in mind, therefore, that two measurements must always be carried out when working with this method. The vessel is filled first with a substance of known susceptibility;

under given conditions the magnetic pull is found to be n milligrams. The same vessel is filled with the unknown substance and under the same conditions the pull is found to be m milligrams. Then, $(n - m)$ is proportional to the difference between the susceptibilities of the known and the unknown substances. The proportionality factor is obtained by measuring $(n - m)$ for two substances, both of known susceptibility.

2. Calibration and Field Strength

Two substances of suitable and known susceptibilities useful as standard substances for the calibration of the proportionality factor are water and air.

Susceptibility is an extensive property and so depends on the amount of the substance. The susceptibility of one cubic centimeter of the substance is called the volume susceptibility, κ . The susceptibility of one gram is the specific susceptibility or mass susceptibility, χ ; the susceptibility of one mole (provided we are dealing with a pure chemical compound and not a mixture) is the molar susceptibility, $\chi_{\text{mol.}}$. In a metal complex compound of high molecular weight, such as hemoglobin, it may be ambiguous to use the concept of "molar" susceptibility, because it may be doubtful whether the smallest molecular unit imaginable, containing one atom of iron, should be considered as the "molecule," or rather its quadruple, which in reality forms the molecular unit. In such a case it is preferable to speak of χ_{Fe} , the susceptibility per gram-atom of iron. The same holds, for instance, for the susceptibility of such preparations as colloidal ferric hydroxide sols, or wet amorphous ferric hydroxide.⁹ In the literature, however, the term "molar susceptibility" is almost always used in the sense of susceptibility per gram-atom of the central heavy metal of the complex.

The volume susceptibilities for air and water in c.g.s.u. are at room temperature: for air, $\kappa = +0.0294 \times 10^{-6}$; and for water, $\kappa = -0.720 \times 10^{-6}$. More precisely:

For dry *air* at absolute temperature, T , and at 760 mm. barometric pressure:

$$\kappa_T = \frac{2.52 \times 10^{-3}}{T^2}$$

That is, at a temperature of 20° C., $\kappa = 0.0294 \times 10^{-6}$; at 24°, 0.0286×10^{-6} ; and at 27°, 0.0300×10^{-6} .

For *water* $\kappa = -0.720 \times 10^{-6}$ according to Seve¹⁰; -0.721×10^{-6} according to de Haas and Drapier¹¹; -0.719×10^{-6} according to Piccard and Devaud¹²; and -0.722×10^{-6} according to Auer.¹³

⁹ L. Michaelis, C. D. Coryell, and S. Granick, *J. Biol. Chem.*, **148**, 463 (1943).

¹⁰ P. Seve, *Ann. chim. phys.*, **27**, 189, 425 (1912).

¹¹ W. J. de Haas and P. Drapier, *Ann. Physik*, **42**, 673 (1913).

¹² A. Piccard and A. Devaud, *Arch. sci. phys. nat.*, **2**, 455 (1921).

¹³ H. Auer, *Ann. Physik*, **18**, 593 (1933).

Air is slightly paramagnetic, due to the oxygen; pure water is diamagnetic. (The effect of a residual trace of dissolved oxygen in water, if approximately but not entirely deaerated, is negligible for most purposes.) The difference is 0.749×10^{-6} . So, when the difference between the magnetic pulls on water and on air is found to be n mg., each milligram of weight corresponds to an increment of susceptibility amounting to (0.749×10^{-6}) per n c.g.s.u. of volume susceptibility, once and for all, for the conditions of the instrument.

This calibration may be checked by comparison with results from other standard substances of known susceptibility. A few standard solutions may be mentioned, of sufficiently different order of magnitude in susceptibility. Using the value for nickel chloride obtained by Nettleton and Sugden,¹⁴ and using Curie's law, which is certainly legitimate for the very small temperature range over which the calculation is extended here, the difference between the volume susceptibilities of a 0.1 *M* solution of nickel chloride and of water, is shown in the accompanying table.

MAGNETIC SUSCEPTIBILITIES OF 0.1 *M* NICKEL CHLORIDE

Temperature, ° C.	κ
18.....	0.0446×10^{-6}
20.....	0.0443
22.....	0.0440
24.....	0.0437
26.....	0.0434
28.....	0.0432

The solution can be readily made up from good crystals of $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ which when stored at room temperature are quite reliable as to the content of water of crystallization. Another good check is a 10% solution of good crystals of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$. The difference between the volume susceptibility of this solution and that of water is shown in the following table.

MAGNETIC SUSCEPTIBILITIES OF 10% CUPRIC SULFATE

Temperature, ° C.	κ
17.....	$+0.642 \times 10^{-6}$
20.....	0.636
23.....	0.630
26.....	0.624

Another good check is a solution of ferric chloride. It has the disadvantage that its iron content must be checked by chemical analysis, but

¹⁴ H. R. Nettleton and S. Sugden, *Proc. Roy. Soc. London*, **A173**, 313 (1939).

the advantage that its susceptibility can be precisely calculated from the spin of the five unpaired electrons of the ferric ion since there are no orbital contributions. Its solution, preferably between 0.05 and 0.02 M , must be made up with 0.01 M HCl as a solvent to prevent hydrolysis. The difference between the volume susceptibility of a 0.0100 M solution of FeCl_3 and that of water is 0.146×10^{-6} c.g.s.u. at 298°K . ($= 25^\circ \text{C}$.) and is proportional to the concentration and, for all practical purposes within the required temperature range, inversely proportional to the absolute temperature.

The unknown substance to be measured is filled into the glass cylinder either in the form of a finely ground powder, or in the form of a liquid, either pure or a solution. The volume susceptibility of the substance measured in this way, κ_v , is independent of the volume capacity of the vessel. It is the susceptibility of one cubic centimeter of the filling. It is this volume susceptibility which is always primarily measured and is the basis for all further calculations.

The methods for measuring magnetic susceptibility described in this chapter are all relative, comparing the effect of a given magnetic field upon an unknown substance with the effect of the same magnetic field upon a reference substance of known susceptibility. Therefore it is never necessary to measure the magnetic field strength, and the methods for its determination need not be discussed here in general. Suffice it to mention one method, which needs no other experimental data than those obtained from the calibration of the vessel with water and air. Let us suppose the difference in pull between water and air has been found to be in milligrams, $= \Delta$. Then the field strength, H , expressed in gauss, in the homogeneous part of the field, where it has its maximum, is:

$$H = \sqrt{\frac{2\Delta}{a \times 0.749 \times 10^{-6} \times 1.019}} \quad (3)$$

where a is the amount of water, in grams, contained in a column of 1 cm. height, in the cylindrical vessel. The factor 1.019 converts milligrams into dynes. The factor 0.749×10^{-6} is the difference between volume susceptibilities for water and air. For instance, when $\Delta = 25$ mg. and $a = 1.00$ g., $H = 7150$ gauss. Field strengths used in the methods described may vary between 1000 and 15,000 gauss or more.

3. Compensation Vessel

The method just described is based on the difference in weight between two measurements. This difference sometimes may be a relatively small magnitude, obtained as a difference of two large magnitudes, and so may

often be liable to a relatively large experimental error. The following modification of the vessel, first used by Freed and Kasper,¹⁵ reduces the influence of this experimental error. The real advantage of this method manifests itself especially on using the "deflection method" to be described below. Instead of a single cylindrical vessel, a double vessel is used, or a cylinder of uniform cross section, double the length of the single one, with a glass septum dividing it into two compartments (Fig. 2). The upper compartment is filled with the unknown solid or solution; the lower is filled with air (for a solid) or (for a solution) with a liquid of approximately the same susceptibility as that of the pure solvent; and the double vessel is suspended so that the septum is approximately in the center of the homogeneous field between the pole pieces of the magnet. When the substances in the two compartments are of nearly the same susceptibility, the pull of the magnetic field is in opposite directions and is nearly cancelled. When the contents of the lower compartment are kept unchanged, and the contents of the upper compartment are varied, the difference in susceptibility is obtained as a difference of two magnitudes one of which is almost zero, whereby the limits

Fig. 2.—Compensation vessel.

of error are considerably diminished. On working with aqueous solutions it is advisable to fill the lower compartment, not with pure water, but with a 2-3% agar gel, which has almost the same susceptibility as water and prevents the rising of air bubbles. Another way of avoiding air bubbles and of allowing for expansion or dilatation at very high or very low temperatures is by the use of a gas trap such as is shown in figure 16c (page 1918), at the lower end of the lower compartment.

4. Gouy's Method

Out of the great number of methods that have been used for the measurement of susceptibility, only one will be described in detail. Usually referred to as Gouy's method,¹⁶ it seems to be the most convenient for the purpose. The

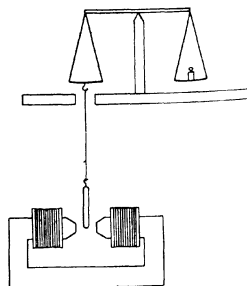


Fig. 3.—Balance, suspension, vessel, and magnet.

¹⁵ S. Freed and C. Kasper, *J. Am. Chem. Soc.*, **36**, 1002 (1930).

¹⁶ L. G. Gouy, *Compt. rend.*, **109**, 935 (1889).

equipment consists of an electromagnet, a balance, a suspension and the cylindrical vessel, a generator of direct current, an adjustable resistance, and an ammeter. Figure 3 is a schematic presentation of the equipment.

A. ELECTROMAGNET AND VESSEL

Designs for the construction of a suitable electromagnet are given by Boas and Pederzani,¹⁷ by du Bois,¹⁸ and by Shaw.¹⁹ The soft iron used as a core should possess as little residual magnetism as possible so that shortly after breaking the current the field strength, at least within the limits of error, can be considered as zero. A commercially available electromagnet is the "Isthmus" magnet (Fig. 4) (*General Electric Co.*). The field strength attainable with the latter, without damage to the instrument, is not quite so high as with the others, but is sufficient for most purposes.

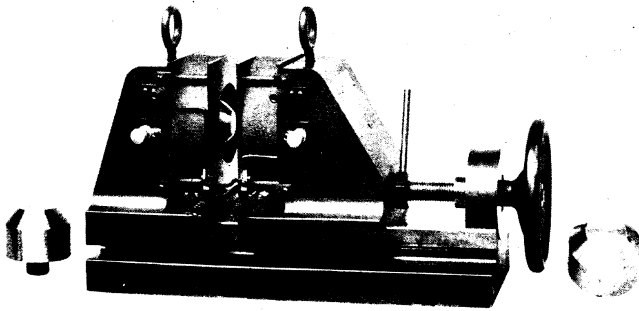


Fig. 4. Isthmus electromagnet (courtesy *General Electric Co.*).

From the curves in figure 5, it may be seen that, for pole gaps not smaller than 1 cm., the field strength is accurately proportional to the amperage up to ten amperes. The magnet is equipped with a cooling system, either for water or air. In redesigning this magnet, two items should be taken into consideration: to increase somewhat the limit of amperage which the instrument can stand without damage (it is now ten amperes); and to raise somewhat the position of the pole pieces to allow a greater clearance between the pole pieces and the floor. As it is now, there is just enough

¹⁷ H. Boas and T. Pederzani, *Z. Physik*, **19**, 351 (1923).

¹⁸ H. du Bois, *Z. Instrumentenk.*, **31**, 362 (1911).

¹⁹ E. J. Shaw, *Rev. Sci. Instruments*, **2**, 611 (1931).

space for a double vessel of sufficient length, but it may be desirable to have somewhat more space.

High sensitivity is usually desirable. This is effected by making the pole gap as narrow as is compatible with the free movement of the cylindrical vessel. The vessel should be at least 2 mm., or rather more, distant from each pole piece. On working with the device for low temperature, (described on page 1916), the distance must, of course, be greater.

In regard to the influence of the width of the pole gap and the dimensions of the vessel upon sensitivity, the following remarks will clarify the situation. In figure 6, b is the diameter of the vessel and a is the distance

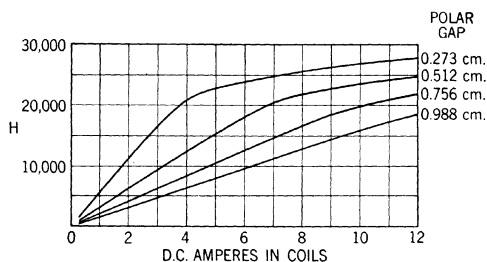


Fig. 5.—Calibration curves on Isthmus electromagnet with pole face of 3-cm. diameter and 1280 total turns in coils (courtesy *General Electric Co.*).

between the vessel and the pole piece at each side. When the width of the pole pieces and the diameter of the vessel are changed so that the ratio of a to b is kept constant, the sensitivity is unchanged. With smaller dimensions the field strength is increased, but the amount of material in the vessel is decreased in the same proportion. Since there is a practical limit in decreasing a , a higher sensitivity can be obtained with a vessel of larger cross section. But the advantages would be really appreciable only for very large vessels, which, however, are more difficult to operate and require very much material. A cross section of 0.5 to 1 cc. of the vessel with a pole gap so that a is about 1 to 2 mm. affords sufficient sensitivity for most purposes, although the higher sensitivity attainable with vessels of 2 or 3 cm. diameter may be advantageous if abundant material is available. With the smaller vessel, the amount of material necessary may be around 2 cc. if liquid, or from 0.3 to 2 g. for solids, depending on the density and the particular kind of packing. For measurements on solid materials, the volume of the cylinder from the bottom (or from the septum when the double vessel is used) up to a mark close to the brim is calibrated. The

vessel is weighed first empty, then after it is filled with the solid material. The material should be very finely ground and packed into the vessel as homogeneously as possible. A good way of doing this is by filling in the material in portions and each time tapping the vessel on the table until its volume appears to be constant.

B. BALANCE AND SUSPENSION. MEASUREMENT BY WEIGHT AND BY DEFLECTION

The pull is measured in terms of change in weight. The writer has made use of a slight modification of the method which has the advantage of a high sensitivity, so that the results in general can be obtained at lower field strengths than with the other method without impairing their accuracy. Instead of compensating the magnetic pull by weights, the pull is measured by watching the maximum deflection on a microscopic scale fastened at the pointer of the balance. The scale has 200 divisions, each corresponding to a deflection caused by approximately 0.01 mg. The total magnetic pull in any experiment is kept smaller than about two milligrams, which would correspond to a deflection over the whole length of the scale, and should be possibly between 0.7 to 1.5 mg. Since the total deflection must always be kept within the range of this scale, the double vessel (compensation vessel) described above must be used. In order to attain a pull within the desired range, the amperage is adjusted for each measurement adequately, and the deflection always recalculated for one standard amperage, say ten amperes. This requires a knowledge of the relationship of amperage to magnetic pull for the actual conditions of the instrument. For an electromagnet with iron core, it is difficult to foresee this relationship in general. It must be calibrated for each instrument. For low amperage, the field strength will be, in general, proportional to the amperage. For the Isthmus magnet, the field strength is proportional to the amperage up to ten amperes for a pole distance not smaller than one centimeter. Since the magnetic pull is proportional to the square of the field strength, the pull is proportional to the square of the amperage under the conditions just mentioned.

The balance should be of sufficient sensitivity, and magnetically damped. Although microbalances of special construction have been recommended, it is more practical, in the writer's opinion, to use a semimicrobalance with magnetic damping by means of a small permanent horseshoe magnet between the poles of which a thin aluminum plate, attached to the end of one arm of the balance, is located. The position of the magnet should be ad-

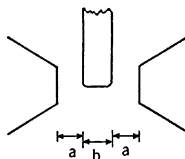


Fig. 6.—Vessel between pole pieces.

justable, and adjusted so as to bring about critical (or very nearly critical) damping. It is necessary that the changing of weights, within sufficiently large limits, be accomplished without opening the case, and, within reasonable limits, even without arresting the balance, as can be done with the chainomatic balance. Usually the reproducibility under the rather severe conditions of a magnetic measurement with the deflection method is within one or two divisions of the microscopic scale mentioned above. On taking the average of several successive readings, a fairly high accuracy can be attained.

To the lower surface of one pan of the balance (Fig. 3) a hook is attached from which the cylindrical vessel previously described (page 1894) is suspended through a hole in the floor of the balance. The suspension, at least 30 cm. long, may be a light silver chain, or a thin wire of aluminum or copper. At the lower end of the suspension is a hook to which the cylindrical vessel can be conveniently attached and detached. The vessel is suspended so that its lower end (for a simple cylinder), or its septum (for a double vessel), hangs in the center between the pole pieces of the magnet. A suitable diameter for the outer circular surface of the cone-shaped pole pieces is 3 cm., if the cylindrical vessels are of the dimensions given before.

C. GENERATOR AND RESISTANCE

In addition to a generator for direct current, possibly one that is not used for other purposes simultaneously, in order that the current be constant, a suitable set of adjustable resistances to vary the current from ten (or more) amperes to less than one ampere is needed. An ammeter and a switch should be so located that they are controllable by the operator at the balance.

Another device²⁰ for keeping the current intensity constant, even without water cooling, is an automatic current regulator. The two ends of a resistance of 0.01 ohm, inserted in the circuit, are shunted and the potential difference at the two ends of the resistance is automatically regulated by a photoelectric cell device. Change in current intensity due to heating during the measurement is conspicuous only with high current intensity and need not often be considered with such low current intensities as are usually used for the "deflection method." It is justifiable to assert that the limiting factor with respect to accuracy and reproducibility is the constancy of the amperage during the measurements.

D. ASSEMBLY AND OPERATION

The whole system must be carefully protected from air currents. For operations at room temperature, the following device is simple and suffi-

²⁰ P. W. Selwood, *Magnetochemistry*. Interscience, New York, 1943.

cient. The suspension wire is surrounded by a wooden box which has the front wall on hinges for opening and closing. The upper end of the box reaches up to the lower surface of the floor of the balance case. The space from around the top of the box to the lower surface of the balance case is surrounded by a mantle, as of cardboard protected by thick layers of cotton all around. An easy and adequate way of protecting the lower part of the system, including the space between the poles of the magnet, is by wrapping it tightly with windproof cloth, or better, by closing the open gap in the rear with Plasticin, and in front, where it must be readily removable, with a stick of wood with a felt lining. This simple device allows one to work with very narrow pole gaps so that high forces are exerted even at low amperage. However, it can be used only for measurements at room temperature.

The cylindrical vessel must be suspended in the center between the pole pieces. Although a mechanical device for small horizontal displacements of the balance is most satisfactory, there is no difficulty in carrying out slight displacements of the balance by hand. No ultimate precision in centering the position is required, provided the lower end of the vessel (or the septum of the double vessel) is within the homogeneous part of the field.

For the deflection method, only the double, or compensation, vessel can be used because the total deflection must be, for a reasonable amperage, within the range of the microscopic scale. In general, a current within the range of 0.8 to 10 amp. will be adequate. To measure the deflection, the vessel is tared by weights so that the equilibrium position of the pointer, in the absence of the magnetic field, is at some convenient, arbitrary point of the microscopic scale. Then the current switch is abruptly closed and the maximum deflection is read. With the writer's instrument, the maximum position is reached after about 15 sec. Then the resistance is adapted to produce a deflection, if possible, of about 75 to 150 lines, equivalent to about 0.75 to 1.5 mg., the amperage is read on the ammeter, and the current is broken. The deflection of the back-swing is not considered because it is not reproducible. Ample time is allowed for the pointer to come to rest again. It does not matter if this zero point happens to be somewhat different from the original one. Then the whole procedure is repeated. As a rule, depending upon the degree of accuracy desired, one may take the average of 5 to 10 readings. They should not differ from each other by more than $\pm 2\%$ of the average value. The deflection in terms of scale divisions is next recalculated for one arbitrarily chosen standard amperage, *e. g.*, ten amperes. The measurements are made successively without varying the content of the lower compartment (water or air, according to conditions) but changing the contents of the upper compartment from the

reference substance (water or air) to the material to be determined. The difference between the number of lines of deflection, valid for ten amperes, of the unknown object and the standard object is converted into the difference between the volume susceptibilities of the two objects by multiplication with a proportionality factor which is established similarly as for the other method. The deflection with air in the upper compartment, re-

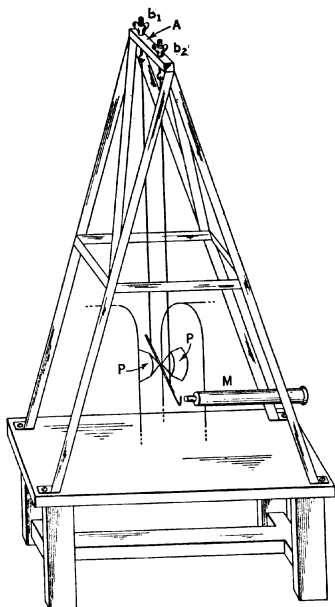


Fig. 7.—Theorell's magnetic deflection balance.²¹

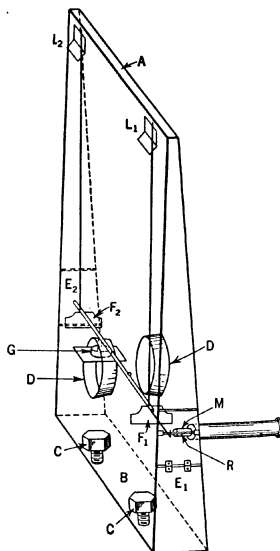


Fig. 8.—Details.²¹

calculated for ten amperes, is algebraically subtracted (with proper regard for the signs of the deflection) from the deflection with water in the upper compartment, also recalculated for ten amperes. This difference is equivalent to an increment of volume susceptibility of 0.749×10^{-6} c.g.s.u. The meaning of one line of deflection (at 10 amp.) in terms of volume susceptibility is thereby obtained. This figure may be called the "vessel constant." In vessels of the dimensions given on page 1896 for the magnet used by the writer, one line of deflection at ten amperes is usually equivalent to an increment of volume susceptibility of 2 to 5×10^{-10} c.g.s.u.

²¹ H. Theorell, *Arkiv Kemi Mineral. Geol.*, A16, No. 1, 1-8 (1943) (in English).

This method has been found by the writer to be so convenient and reliable that it may be recommended as a routine method of nearly general application for measurements at room temperature. In general, the final result, in terms of volume susceptibility, may be said to be reproducible to $\pm 1\%$ under not too unfavorable conditions. A higher accuracy has

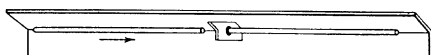


Fig. 9.—Brass angle for Theorell's magnetic deflection balance.²¹



Fig. 10.—Further details for Theorell's magnetic deflection balance.²¹

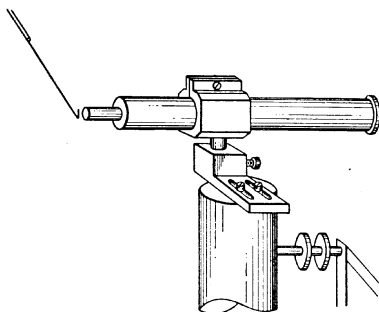


Fig. 11.—Details of observation microscope for Theorell's magnetic deflection balance.²¹

been claimed for some methods by some authors, but seems difficult to attain.

Other types of deflection methods, such as that of Theorell,²¹ have been used. Theorell places the vessel in a horizontal, instead of perpendicular, position between the poles of the magnet. The following description of this apparatus is, in part, a verbatim quotation of the original.

The tube that is suspended in the magnetic field is made as follows. A thin-walled glass tube with a diameter of about 10 mm. is drawn out until a tube with an outer diameter of 2.6–2.7 mm. is obtained; two lengths of 110 mm. are cut off. One of the tubes is partially filled with acetone and pressed against a circular celluloid disk, about 4 mm thick. When it is firmly stuck, the tube is placed in a brass angle

that is provided with a hole as shown in figure 9 so that the firmly gummed celluloid disk lies freely in the hole. The disk is moistened with acetone on the outside and one end of the other tube is pressed against it. It is held in position with modeling clay during the process of drying. The superfluous celluloid is wiped off with acetone. In this way a straight tube with a transverse partition is obtained, as a substitute for the all-glass "double vessel" previously described (page 1894). A thin, short glass rod, *B* (Fig. 10), is attached to this tube by means of a celluloid-acetone solution. At the other end a very fine glass rod, *C*, is attached, which is bent at the end at right angles as in *D*. This vertical end is the "indicator" to be observed with the microscope. Furthermore, a little hook, *E*, is attached with celluloid.

The stand of the instrument is made of brass. Four supports, attached in pairs at their upper ends, bear a horizontal beam, *A*, a little over one meter long above the center of the pole shoes, *P* (Fig. 7). In *A*, there are two vertically adjustable screws, *b*₁ and *b*₂ (by means of which the hooks from the two suspending hairs are suspended), which may be raised or lowered for the adjustment of the position of the tube between the pole shoes. The stand is stabilized with transverse and diagonal brass beams. The housing around the hair-suspended tube is made of 2 mm. thick celluloid, stuck together with acetone at the corners. The housing is open at the top. This opening is closed by suspension beam *A* (Fig. 7). The floor of the housing, *B* (Fig. 8), rests upon screws, *C*, in the base plate of the magnet so that its height may be adjusted. In each of the sides there is a round hole with circular washers, *D*, which fit airtight around the poles of the magnet. Front and back there are openings (*E*₁ and *E*₂) to enable one to get at the tube. Inside *E*₁ is stretched a rubber band, *R*, in which the tube is held when the experimental solution is to be introduced. On the knife edges, *F*₁ and *F*₂, which are attached with celluloid and are made of Plexiglas, the tube may be stabilized or released. The bowl, *G*, contains glycerin which should be changed daily when measurements are being made. Herein is immersed the needle brake, *B* (Fig. 10), which serves as a damp to the swings of the tube. Through the hole, *M* (Fig. 8), provided with a rubber washer, is introduced the objective of the microscope (270× magnification with scale divisions in the eyepiece). Through doors *L*₁ and *L*₂ one may reach the suspending hooks of the hair strands.

The author used a large electromagnet of the du Bois type, the surface of the pole ends being 9 mm. in diameter and the pole gap 5.5 mm. The observation microscope is mounted very firmly as shown in figure 11. When the support for the suspension of the tube has been screwed fast to the table, the housing is set in place while the poles of the magnet are drawn apart. Two separate strands of hair are knotted, each one meter long, with loops at each end. In one of the loops in each strand is hung a weight that is introduced through the openings, *L*₁ and *L*₂ (Fig. 8). The upper loops are hung on the hooks under the screws *b*₁ and *b*₂ (Fig. 7). The tube is then passed through the two lower loops. The magnet poles are moved into position and adjusted to the desired distance (5.5 mm.). The position of the tube is shifted so that the partition wall is in the center of the magnetic field and the hair strands are parallel to each other. The hair loops are fixed to the tube with a celluloid-acetone solution. The tubes are now filled: first both ends

are filled with water; later on the water of one half is replaced with the solution to be measured—the other remains permanently filled with water, or any other solution of convenient susceptibility, as reference. The filling of the end which is turned toward the microscope takes place as follows. The two rubber bands, *R* (Fig. 8), are separated with tweezers; the tube is drawn out with a hook of copper wire and released from the grip of the rubber bands. The other end, which is to contain the permanent filling, may be filled either in the same manner, or simply by holding it between thumb and finger. Its end is sealed with a drop of paraffin oil to prevent evaporation. Bowl *G* (Fig. 8) is filled with glycerin, the tube is released, and damping brake *B* (Fig. 10) is lifted into the bowl.

When taking the measurements the water value is determined first. In doing this the position of the tube may be corrected. From now on, calibration of the vessel and the measurements proper are carried out according to the same principles as in the "deflection" method described above, except for the fact that air cannot be used as the reference for calibration.

Lewis and Calvin²² have modified the method for the study of the paramagnetism of the fluorescent state of organic molecules.²³ Instead of Theorell's housing, their housing consists of a vertical glass tube for the suspension and a horizontal glass tube for the horizontal bar, each 2 cm. in cross section. This device not only affords protection from drafts but also allows replacement of the air by a gas other than air, and of a pressure other than one atmosphere. With a total suspended weight of 0.14 g., a horizontal displacement of 1 μ is produced by a force of 0.1 μ g. Such high sensitivity is attained at the expense of accuracy, but it serves very well the particular purpose of the authors.

E. EXAMPLE OF MEASUREMENT

The following actual measurements were performed with the "deflection method" described on page 1897. The magnetic pull is expressed in terms of lines of the microscopic scale at the pointer of the balance. The measurements were carried out at room temperature.

Calibration.—The pole gap is set to approximately 1.3 cm. A cylindrical double vessel is used. The upper compartment of the cylinder is 10.5 cm. long; the outer diameter is 7 mm. The capacity, from the septum up to a mark slightly below the top, is 2.075 cc. Both compartments are filled with air. The deflection at 7.50 amp. is -28.5 lines, as an average of ten readings, which deviate from the average value in general by not more than ± 1 line. Since the pull is, under the conditions of the instrument, proportional to the square of the amperage, the pull at 10 amp., which is chosen as standard, is:

²² G. N. Lewis and M. Calvin, *J. Am. Chem. Soc.*, **67**, 1232 (1945).

²³ G. N. Lewis and M. Kasha, *J. Am. Chem. Soc.*, **66**, 2100–2116 (1944); **67**, 994–1003 (1945).

$$-\frac{28.5(10.0)^2}{(7.50)^2} = -51 \text{ lines}$$

Vessel Constant.—The lower compartment is again filled with air and the upper with pure water. The deflection, measured at 2.36 amp., recalculated for 10 amp., is -2432 lines.

The difference between volume susceptibilities, κ , for water and air is 0.749×10^{-6} . This corresponds to $-2432 - (-51)$, or -2381 lines. Hence one line of deflection at 10 amp. signifies an increment of volume susceptibility equal to:

$$\frac{0.749 \times 10^{-6}}{2381} = 3.14 \times 10^{-10} \text{ c.g.s.u.}$$

This is the "vessel constant."

Susceptibility of Solid Potassium Ferricyanide.—Finely ground powder of the substance is placed in the upper compartment and air in the lower. The weight of the empty vessel is 18.5655 g.; the weight after filling with the powder to the mark near the top is 21.1809 g. Hence, the weight of the powder is 2.6154 g. Since the capacity of the vessel is 2.075 cc., the amount of substance in 1 cc. of the vessel is 1.260 g. The deflection measured at 0.745 amp. and recalculated to 10 amp., is $+26,550$ lines. So:

$$\begin{array}{ll} \text{the powder gives} & +26,550 \text{ lines} \\ \text{and air gives} & -51 \text{ lines} \end{array}$$

Hence, the difference between the susceptibility of 1 cc. of the powder and that of 1 cc. of air is represented by $+26,601$ lines, which corresponds to an increment of volume susceptibility equal to $26,601 \times 3.14 \times 10^{-10} = +8.35 \times 10^{-6}$ c.g.s.u. For air, $\kappa = +0.029 \times 10^{-6}$ c.g.s.u.; hence, κ for 1 cc. of the powder equals $+8.32 \times 10^{-6}$ c.g.s.u.

The powder contained in 1 cc. of the cylinder consists of 1.260 g. or (density taken $= 1.84$) 0.685 cc. of solid substance, and 0.315 cc. of air. These 0.315 cc. of air contribute $+0.315 \times 0.029 \times 10^{-6} = +0.0091 \times 10^{-6}$ c.g.s.u. Hence, the 1.260 g. of the substance has a susceptibility $= (8.32 - 0.009) \times 10^{-6} = +8.31 \times 10^{-6}$ c.g.s.u. Hence, the susceptibility per gram, $\chi = +6.60 \times 10^{-6}$ c.g.s.u., and (taking the molecular weight $= 329$), $\chi_{\text{mol.}} = 2180 \times 10^{-6}$ c.g.s.u.

This is the combined paramagnetic and diamagnetic susceptibility. According to Pascal's rule (see page 1908), disregarding the iron atom, the diamagnetic susceptibility of potassium ferricyanide is -143×10^{-6} c.g.s.u. Hence, the corrected, purely paramagnetic, molar susceptibility is 2323×10^{-6} c.g.s.u.

The experiment was performed at 23°C. (296°K.). The effective magnetic dipole moment is (in Bohr magnetons):

$$\mu_{\text{eff.}} = 2.84\sqrt{\chi_{\text{mol.}}T} = 2.34$$

The term "effective moment" signifies that it has been calculated according to Curie's law, assuming $\vartheta = 0$. The true moment may be slightly different if $\vartheta \neq 0$, and the Curie-Weiss law is used (see page 1915).

F. MAGNETIC TITRATION

If a substance of given molar susceptibility reacts with some reagent which is magnetically relatively indifferent so as to be converted into another substance of different susceptibility, a magnetic titration can be accomplished. The method may be characterized by the example of the titration of hemoglobin compounds according to Coryell, Pauling, and Dodson.²⁴ Oxyhemoglobin is diamagnetic; reduced hemoglobin is paramagnetic. A solution of oxyhemoglobin is titrated with sodium dithionite, $\text{Na}_2\text{S}_2\text{O}_4$ (sodium "hydrosulfite") which reduces it to hemoglobin. After each addition of a portion of the dithionite, the susceptibility is measured (Fig. 12).²⁴ The titrations were performed as follows. The cylindrical tube containing the oxyhemoglobin solution was closed with a rubber cap and portions of dithionite of suitable concentration solution, respectively, were injected from a syringe with a fine canula through the rubber cap. A small correction for the effect of the added reagent, which is diamagnetic, is applied for the Δw values. This correction is either established by a separate measurement of the reagent or calculated according to Pascal. It is assumed that the diamagnetic susceptibilities of dithionite and of its oxidation products are the same, which is certainly true within the limits of experimental error, especially in consideration of the smallness of the correction. Provided the difference between the molar susceptibility of reduced hemoglobin and that of oxyhemoglobin has been established before, such a titration made with a solution of unknown concentration can be used for a quantitative determination of hemoglobin. The linearity of the titration curve indicates clearly the fitness of the procedure. There is a sharp end point of titration. The method has been used also for other derivatives of hemoglobin and should certainly be useful in other instances.

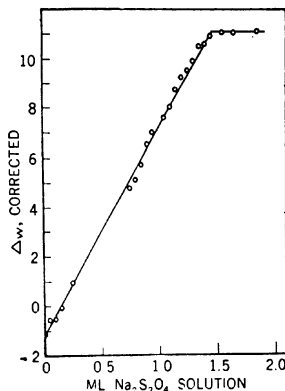


Fig. 12.—Magnetic titration of oxyhemoglobin with sodium dithionite at pH 6.75.²⁴

²⁴ C. D. Coryell, L. Pauling, and R. W. Dodson, *J. Phys. Chem.*, **43**, 825 (1939). See also: L. Pauling and C. D. Coryell, *Proc. Natl. Acad. Sci. U. S.*, **22**, 210 (1936); C. D. Coryell, F. Stitt, and L. Pauling, *J. Am. Chem. Soc.*, **59**, 633 (1937).

5. Methods Other Than That of Gouy

Only a few of the numerous methods for the measurement of magnetic susceptibility other than Gouy's will be briefly mentioned.

The Curie-Cheneveau²⁵ Balance.—The pole shoes of a permanent magnet are given the shape of truncated wedges. The object, in the shape of a cylinder, is placed between the pole pieces so that the whole cylinder hangs between the wedges, while in the Gouy method the cylinder protrudes above the pole pieces. The magnet is then moved forth and back with respect to the sample, so that the whole cylinder is in the inhomogeneous field. The object automatically locates itself in the region of maximum attraction or repulsion, namely, that region where $\partial H/\partial X$ has its maximum. The force is measured by a torsion balance. By comparing this force with the force for an object of known susceptibility, a relative value of the susceptibility of the unknown is obtained.

The Quincke²⁶ Method.—This method can be used for liquids only. The liquid is filled into a narrow U-shaped glass tube, of which one end is enlarged to form a reservoir of large diameter. The meniscus in the other, narrow, arm of the tube is placed between the pole shoes of an electromagnet. The displacement of the meniscus on exciting the magnet is measured in terms of the hydrostatic pressure which compensates the displacement. Many modifications of the method have been described. Useful, very sensitive, but cumbersome in practice, is the method of Wills and Hector.²⁷

6. Calculations and Interpretations of Susceptibility

A. CALCULATION OF VOLUME, GRAM, AND MOLAR SUSCEPTIBILITIES

In order to calculate volume, gram, and molar susceptibilities, the amount of material contained in 1 cc. of the cylindrical vessel must be known, *i. e.*, for solutions the concentration must be known. For solids, the weight of the solid divided by the volume capacity of the vessel yields the amount of substance in 1 cc. of the vessel. The total weight is not well reproducible for successive fillings, of course, yet in each case the filling can be made so homogeneous with a little practice that the final result of volume susceptibility is reproducible to within 1 or 2%, depending on the consistency of the powder. For very fluffy powders the reproducibility may be less. Three cases may be distinguished:

(1) The substance is a pure liquid. Then the specific (or mass) susceptibility, χ , is obtained from the measured volume susceptibility, κ , by

²⁵ C. Cheneveau, *Phil. Mag.*, **20**, 357 (1910).

²⁶ G. Quincke, *Ann. Physik*, **24**, 347 (1885); **34**, 401 (1888).

²⁷ A. P. Wills and L. G. Hector, *Phys. Rev.*, **23**, 209 (1924).

dividing it by the density. The molar susceptibility, $\chi_{\text{mol.}}$, is derived from this by multiplication by the molecular weight.

(2) The substance is a finely ground powder. Then, from the density of the solid, one can calculate the volume occupied by the solid and that occupied by air, in 1 cc. of the packing. The contribution by air to the total volume susceptibility is usually very small. It can be calculated from the volume occupied by air, and the susceptibility of 1 cc. of air, which is equal to $+0.029 \times 10^{-6}$ c.g.s.u. The total susceptibility of the packing is corrected for the air content in order to obtain the susceptibility of the unknown solid in 1 cc. of the packing. Its susceptibility per cubic centimeter, κ , or the susceptibility per gram, χ , or per mole, $\chi_{\text{mol.}}$, can then be obtained. This susceptibility is the resultant of the diamagnetic contribution of the solid substance, and the paramagnetic one, if there is any.

(3) The substance is a solution, and the susceptibility of the solute is the desired result. Here we must distinguish between diamagnetic and paramagnetic solutes. (a) If the solute is diamagnetic, the volume susceptibility of the solution will be of the same order of magnitude as that of the solvent. In this case, only solutions of rather high concentration can give accurate results. If the concentration and the density of the solution are known, it is easy to calculate how much solvent and how much solute are contained in 1 cc. When the volume susceptibility of the solution is κ , then the susceptibility, per gram, of the solute, χ , is:

$$\chi = \frac{\kappa - (1 - p)\kappa_{\text{solv.}}}{p} \quad (4)$$

where $\kappa_{\text{solv.}}$ is the volume susceptibility of the pure solvent and p is the mass, in grams, of the solute contained in 1 cc. of the solution. (b) If the solute is paramagnetic, in general the same procedure can be used. Since the effect of a paramagnetic molecular species is very much greater than that of a diamagnetic one, even rather dilute solutions may give fairly accurate results. When the concentration of a paramagnetic solute is $<10\%$ in weight, it is usually correct within the limits of experimental error to assume that the difference between the volume susceptibilities of solution and of the solvent represents the paramagnetic contribution of the solute. We assume that the diamagnetic contributions of the paramagnetic solute and of the solvent displaced by it in the solution are both very small and that their difference is negligible. Then, obviously, the difference between the susceptibilities of solution and solvent is due to the paramagnetic contribution of the solute contained in 1 cc. of the solution. From this, the specific or molar paramagnetic susceptibility of the solute is easily calculated.

B. PASCAL'S DIAMAGNETIC INCREMENTS AND APPLICATIONS OF MEASUREMENTS

The molar diamagnetic susceptibility can be calculated according to Pascal²⁸ by adding the atomic increments, with slight structural corrections. Pascal's figures were based on the assumption that the volume susceptibility for water is -0.75×10^{-6} . Since the newer measurements agree on the value -0.720×10^{-6} , the figures are recalculated in accordance with the more recent value. In table I, all figures are given in c.g.s. in units of 10^{-6} . An example for the calculation of molar diamagnetic susceptibilities follows table I.

TABLE I
ATOMIC DIAMAGNETIC INCREMENTS IN C.G.S. UNITS ($\times 10^{-6}$)

H.....	-2.93	P.....	-26.3
C.....	-6.00	As.....	-43
N (in open chain).....	-5.57	Sb ^{III}	-74
N (in ring).....	-4.61	Bi.....	-192
N (in monoamides).....	-1.54	Li.....	-4.2
N (in diamides or imides).....	-2.11	Na.....	-9.2
O (bonded by single bonds as in alcohols or ethers).....	-4.61	K.....	-18.5
O (bonded by a double bond to C, in addition to another O bonded by a single bond, as in carboxyl).....	-3.36	Mg.....	-10
Ag.....	-31	Ca.....	-15.9
Tl.....	-40	Al.....	-13
Zn.....	-13.5	SOME CONSTITUTIONAL CORRECTIONS	
Hg ^{II}	-33	For:	
Pb ^{II}	-46	Double bond between two C atoms, in aliphatic compounds.....	
F.....	-11.5	Two conjugated double bonds.....	
Cl.....	-20.1	Triple bond.....	
Br.....	-30.6	Each C in aromatic ring.....	
I.....	-44.6	Each C as a member of two aromatic rings.....	
S.....	-15.0	Cl, bound to C.....	
Se.....	-23	Br, bound to C.....	
Te.....	-37.3	I, bound to C.....	

$\chi_{\text{mol.}}$ of hydroquinone, $\text{C}_6\text{H}_4(\text{OH})_2$:

$$6 \text{ C} = -36$$

$$6 \text{ H} = -17.6$$

$$2 \text{ O} = -9.2$$

$$\text{Correction for 6 aromatic C atoms} = -1.4$$

$$\chi_{\text{mol.}} = -64.2 \times 10^{-6}$$

An application of magnetic measurements in which the structural corrections play an important role was found by Farquharson.²⁹ When a substance containing double bonds and able to form polymers is subjected to

²⁸ P. Pascal, *Ann. chim. phys.*, numerous papers from 1910 to 1925.

²⁹ J. Farquharson, *Trans. Faraday Soc.*, **32**, 219 (1936).

magnetic measurement, in the course of a polymerization the diamagnetic susceptibility changes as polymerization goes on due to the disappearance of a double bond and the formation of a new single bond. The authors found that each bond formed manifests itself by a change of susceptibility of equal amount. The magnitude of the effect varies with the particular substance, but for each substance the change is the same for each bond formed. If a dimer is formed according to $2B \rightarrow B_2$, the susceptibility, per mole of dimer, χ_D , is:

$$\chi_D = 2\chi_0 + \lambda \quad (5)$$

where χ_0 is the molar susceptibility of the monomer, and λ a correction term. If n moles of the monomer combine to form the polymer, the susceptibility for one gram of the polymer is:

$$\chi = \frac{n\chi_0 - (n+1)\lambda}{nM} \quad (6)$$

where M is the molecular weight of the polymer. This relationship has been found valid by Farquharson for the polymerization of 2,3-dimethylbutadiene. Figure 13 shows the change of magnetic pull with the Gouy method, in terms of milligrams, plotted against time. On taking into

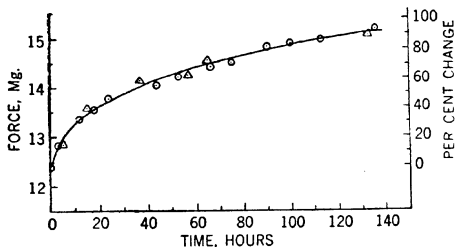


Fig. 13.—Polymerization of dimethylbutadiene.²⁹ ○, Magnetic result. △, Lebedeff's analytical result.

consideration the simultaneous change in density, the change of mass susceptibility can be calculated. On the other hand, the degree of association, in terms of n , at any time during the polymerization, can be calculated with fair accuracy, provided n is not too large. The author has also studied the polymerization of polyhydroxymethylenes. Each step of polymerization consists in adding the group $-\text{CH}_2\text{O}-$. The Pascal constant of this group was found to be -14.9×10^{-6} . This is somewhat different from the Pascal value of >CHOH , which is -16.6×10^{-6} , although from Pascal's rule such a difference cannot be anticipated.

In many cases the mass susceptibility has been found by Bhatnagar³⁰ to be lowered on the formation of a dimer, for instance: anthracene \rightarrow dianthracene; acetone \rightarrow diacetone alcohol; furfural \rightarrow furoin; and others. Polymerization of styrene³¹ and of thiocyanogen³² has been studied similarly. Farquharson and Ady³³ found that 2,3-dimethylbutadiene, when polymerized in absence of any catalyst, first shows a slight decrease, and later an increase, in diamagnetic susceptibility. The authors interpret this by assuming that free radicals (see below) are formed in the first stage of the reaction. It is questionable whether the experimental material obtained so far justifies this interpretation and whether the free radicals, which most likely have been formed as a primary reaction product, will ever accumulate to such an extent as to manifest their presence by paramagnetism of measurable magnitude considering the sensitivity of the methods in their present stage.

7. Free Radicals

In organic chemistry, paramagnetic substances are usually free radicals. In general, free organic radicals are highly autoxidizable in the dissolved state, although they may be more or less stable, sometimes quite stable, in the solid state. If so, free radicals in the solid state can be measured with the methods just described for solid substances. However, the magnetic state may be, and usually is, different in the dissolved state from that of the solid state. A few examples will clarify the situation and show why it is necessary to measure the magnetic property both in the solid and in the dissolved state, if possible.

A. TYPICAL CASES

First Case.—The free radicals of the type triphenylmethyl arise from partial dissociation, *e. g.*, in benzene solution, of a hydrocarbon of the hexamethyl-ethane type. The problem is to measure the concentration of the dissociated part of the ethane, the free radical, for any given total concentration of the ethane in a given solvent at a given temperature. The method has been developed by Müller³⁴ and Roy and Marvel³⁵ and may be described as follows. A known amount of the chloromethane is dissolved in benzene and is converted to the ethane by shaking with silver. If one assumes the reaction is complete and no loss of solvent occurs, the total concentration of the ethane is known. The susceptibility of the

³⁰ S. S. Bhatnagar, M. B. Nevgi, and R. N. Mathur, *Z. Physik*, **100**, 141 (1936).

³¹ S. S. Bhatnagar, P. L. Kapur, and G. Kaur, *J. Indian Chem. Soc.*, **17**, 177 (1940).

³² S. S. Bhatnagar, P. L. Kapur, and B. D. Khosla, *J. Indian Chem. Soc.*, **17**, 529 (1940).

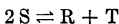
³³ J. Farquharson and P. Ady, *Nature*, **143**, 1067 (1939).

³⁴ E. Müller, J. Müller-Radloff, and W. Bunge, *Ann.*, **520**, 235 (1935). E. Müller and J. Müller-Radloff, *ibid.*, **521**, 89 (1935).

³⁵ M. F. Roy and C. S. Marvel, *J. Am. Chem. Soc.*, **59**, 2622 (1937).

solution is then measured, and, by comparing it with the susceptibility of the pure solvent, the molar susceptibility of the ethane can be calculated in the usual way, using the method described on page 1907. One can then calculate what the diamagnetic molar susceptibility of the ethane should be according to Pascal's rules (page 1908). The apparent molar diamagnetic susceptibility as measured will be smaller than the calculated one, the difference being attributed to the presence of the free radical. Since the molar susceptibility of any free radical in any solvent is always the same, *e.g.*, at 20° C., $+ 1260 \times 10^{-6}$ c.g.s.u., the ratio of the decrement of diamagnetic susceptibility, to 1260×10^{-6} is the molar concentration of the radical. In this way the degree of dissociation of the ethane is established. The accuracy of the method is, of course, impaired if the concentration of the free radical is too low. However, according to the authors quoted, on working with a total concentration of 0.1 *M* of the ethane, the results obtained appear to be rather well reproducible and reasonable, even if not more than about 5% of the ethane is dissociated into radicals.

Second Case.—A substance may be present in the solid state entirely as a free radical, but in solution a dismutation (not a dimerization as in the former case) takes place, as is discussed on page 1772 *et seq.* in the chapter on oxidation-reduction potentials:



An example is Wurster's blue,³⁶ the radical arising from partial oxidation of tetramethyl-*p*-phenylenediamine. According to the equilibrium constant of this reaction, only a certain fraction of the substance, when in the dissolved state, is present as a paramagnetic free radical. Qualitatively, a paramagnetic increment due to the free radical may be detectable and used as evidence of the presence of a free radical. However, due to the low concentrations obtainable of the free radical and to the instability of the system, it is difficult to arrive at quantitative results. Such a system may be more easily studied by potentiometric methods.

Third Case.—A substance which, in the dissolved state, is present in part as a free radical may undergo complete polymerization of the radical in the solid state. In this case, the substance may be paramagnetic in solution, but diamagnetic in the solid state. An example is Wurster's red, in which the radical arises from partial oxidation of asymmetric dimethyl-*p*-phenylenediamine. Here the solution contains the free radical, a diamagnetic species at a higher level of oxidation (the diimine), a diamagnetic species at a lower level of oxidation (the diamine), and a diamagnetic polymerization product of the radical. The crystalline solid is completely diamagnetic and arises from the polymerization of the radical. Qualitatively, Rumpf and Trombe³⁷ have shown that Wurster's red in the dissolved state has a paramagnetic increment caused by the presence of a free radical. However, for the same reason as discussed in the preceding case, the increment is very small, and the stability of the system in solution is not sufficient to allow a quantitative measurement. In any case, the qualitative proof for the radical in crystals of Wurster's red is an important result.

³⁶ L. Michaelis and S. Granick, *J. Am. Chem. Soc.*, **65**, 1747 (1943).

³⁷ P. Rumpf and F. Trombe, *J. chim. phys.*, **35**, 110 (1938).

B. METHOD OF SLOW REDUCTION

In general, in all measurements on free radicals, difficulty is encountered because of the easy oxidation of free radicals when dissolved and, even slightly, exposed to the air. The following method circumvents this difficulty. It can be used to study the magnetic behavior of radicals in the dissolved state, provided the radical is of the type of a semiquinone radical, which can be readily and reversibly obtained by the reduction of a diamagnetic compound in solution. An example is duroquinone^{38,39} in alkaline solution, which can be reduced in two successive univalent steps, of which the first is a free radical in equilibrium with the quinone and the hydroquinone. In such cases, the *method of slow reduction* can be successfully applied, and has the particular advantage that no correction for diamagnetism need be considered at all.

The parent substance of the alleged radical—duroquinone in the above example—is dissolved in a suitable solvent, say a mixture of 80 parts of water with 20 of pyridine or alcohol, containing the desired amount of alkali, acid, or buffer; then a reducing agent is added such that the time of reduction is stretched over a period of one or two hours; and the gradual change of susceptibility of the solution during the time of reduction is measured. At the beginning, the solution will be diamagnetic. During the reduction, the magnetic susceptibility will diminish, *i. e.*, be changed in the direction of paramagnetism; it will reach a minimum, where the paramagnetic effect is at its maximum; and finally the magnetic susceptibility will return to its original diamagnetic state and remain constant. The difference between the maximum and the minimum diamagnetic susceptibilities reveals the maximal amount of free radical which is formed in equilibrium with the quinone and the hydroquinone (Fig. 14).

In order to carry out this method, a reducing agent of a sufficiently sluggish action must be used. At present, only two such agents are available:

(1) When working in alkaline solutions, of about pH 10.5 to 12, glucose can be used at room temperature. Depending upon its concentration, the time of reduction can be adapted to convenience.

(2) When working in slightly acid solutions, of pH 3 to 5, a mixture of methylglyoxal with very little potassium cyanide acts as a sufficiently sluggish reducing agent for redox systems with a normal potential more positive than that of methylene blue. Methylglyoxal can be easily prepared in large quantities.⁴⁰ It is best stored as a 1 *M* aqueous solution in

³⁸ L. Michaelis, M. P. Schubert, R. K. Reber, J. A. Kuck, and S. Granick, *J. Am. Chem. Soc.*, **60**, 1678 (1938).

³⁹ L. Michaelis, *J. Am. Chem. Soc.*, **63**, 2446 (1941).

⁴⁰ G. Hahn and O. Schales, *Ber.*, **67**, 1816 (1934). H. L. Riley, J. F. Morley, and N. A. C. Friend, *J. Chem. Soc.*, **1932**, 1875.

the icebox. The system has thus far been used successfully only with phenanthrene quinone sulfonate in acid solution (pH about 4.6).⁴¹ It could be shown that, with this compound, the intermediate step of reduction in acid solution, which is recognizable by its deep brown color, is not a free radical.

In alkaline solution, methylglyoxal is rapidly converted into lactic acid, which is useless for the purpose. In acid solution, methylglyoxal is stable, but it is slowly oxidized in the presence of potassium cyanide as a catalyst. If no other reducible substance is present, the oxidation of one part takes place at the expense of another part which is reduced. If another reducible substance is present, methylglyoxal simply acts as a reducing agent. The speed of reduction can be regulated by the concentration of the methylglyoxal and especially of the potassium cyanide.

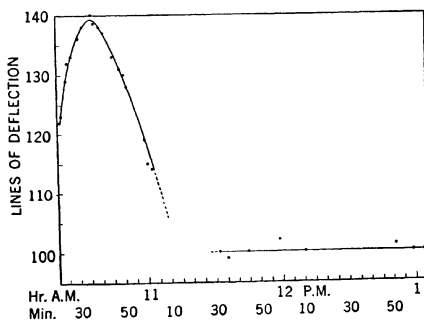


Fig. 14.—Method of slow reduction.⁴⁹

Thus, the method of slow reduction is confined to certain favorable cases, but, in these, it can be used most satisfactorily. The example (Fig. 14) illustrates this method. A solution of 0.232 g. of duroquinone in 10 cc. of a mixture of 4 parts of water and 1 part of pyridine, alkalized with 1.10 cc. of 1.10 *N* sodium hydroxide, was reduced by addition of 80 mg. of glucose. The pull, in terms of lines of deflection, is plotted against the time. One line corresponds to 2.42×10^{-10} c.g.s.u. of susceptibility, or (see below) to 0.000195 mole of a free radical per liter. The total molar concentration of duroquinone (*a*) equals 0.0142; the maximum concentration of the radical (*s*) is that corresponding to 39 lines of deflection and is 0.00757; (*s/a*)_{max.} = 0.53.

The initial value of the susceptibility may not be correct because, at the time of the first measurement, the reduction may have already started. However, the constant final value provides a good reference. The difference between the maximum and minimum values of diamagnetic suscepti-

⁴¹ L. Michaelis, R. K. Reber, and J. A. Kuck, *J. Am. Chem. Soc.*, **60**, 214 (1938).

bility reveals, without any correction, the paramagnetic contribution of the free radical at the time of its maximum concentration. The concentration of the free radical at any time, and especially its maximum value, can be calculated according to the rules discussed below.

With regard to the sensitivity of the method of slow reduction, it may be said that a quantitative determination of the maximum amount of free radical in equilibrium with other, diamagnetic, molecular species is possible if the maximum concentration of the free radical is not smaller than 0.01 molar. Semiquantitative estimates may be obtained at slightly lower maximum concentrations.

C. CALCULATION OF CONCENTRATION OF RADICALS

The paramagnetic contribution to the susceptibility of a free radical is due to the spin of the unpaired electron, as was first pointed out by Lewis.⁴² Paired electrons have no magnetic effect because they have opposite spins. It has been shown that the orbital motion of the electrons has no paramagnetic effect in molecules of the type dealt with in organic chemistry.⁴³ According to quantum mechanics the paramagnetic effect of n unpaired electrons in a molecule can be stated by saying that the magnetic dipole moment, μ , for one mole, expressed in Bohr magnetons (B.M.), amounts to:

$$\mu = \sqrt{n(n+2)} \quad (7)$$

For a radical with a single unpaired electron this is, in B.M.:

$$\mu = \sqrt{3} = 1.73 \quad (8)$$

The Bohr magneton, which is now generally used as the unit of magnetic dipole moment is, with sufficient accuracy, five times (precisely 4.95) that of the Weiss magneton previously used. The Bohr unit was originally defined by using the old Bohr model of the hydrogen atom, having a nucleus and an electron revolving around it in a circular orbit. This orbit is equivalent to a loop of electric current which is equivalent to a magnet with its dipole moment (= the Bohr unit) perpendicular to the plane of the loop. The Bohr model has been discarded and, according to the new quantum mechanics, the orbital motion of the electron in the ground state of the hydrogen atom (as an s electron) produces no magnetic moment at all. The Bohr magneton may now be interpreted as the magnetic moment produced by the orbital motion of a p electron, or the moment produced by

⁴² G. N. Lewis, *Valence and Structure of Atoms and Molecules*, Chemical Catalog Co., New York, 1923, p. 148; *Chem. Revs.*, **1**, 231 (1924).

⁴³ J. H. Van Vleck, *Electric and Magnetic Susceptibilities*. Oxford Univ. Press, New York, 1932.

the spin of one unpaired electron. It may be recalled that, though the *magnetic* moments due to the orbital motion of a *p* electron or due to spin of an electron are equal, the *mechanical* angular moment of the spin is half that of a *p* orbital. Furthermore, although the magnetic effect of an orbital appears in this *definition* of the Bohr unit, orbital effects are completely quenched in organic compounds.

The molar permanent dipole moment, μ , can be calculated from the molar paramagnetic susceptibility, χ , as follows (in B.M.):

$$\mu = 2.84\sqrt{\chi T} \quad (9)$$

T is the absolute temperature and 2.84 the numerical value of the constant $\sqrt{3R/N}$, R being the gas constant, and N Avogadro's number. Equation (9), which shows how the paramagnetic susceptibility of a substance with a given magnetic moment depends on the temperature, is called Curie's law.

Curie's law is usually not strictly obeyed but is replaced by the Curie-Weiss law:

$$\mu = 2.84 \sqrt{\chi(T - \vartheta)} \quad (10)$$

where ϑ is the "Weiss constant," or "field constant." It arises from the mutual interaction of the molecular dipoles. Equation (10) is fulfilled for temperatures which are greater than, and not too close to, ϑ . The measurement of the susceptibility at various temperatures reveals the value of ϑ .

Although, in general, ϑ may vary widely according to the chemical nature and the state of the substance and may even be negative, it is always very small for free organic radicals. The values of ϑ in free radicals so far obtained by comparative measurements at different temperatures are tabulated by Klemm.⁴⁴ They are all very small compared with T , if T is in the neighborhood of room temperature, and the deviation from Curie's law is certainly never great enough to invalidate the following calculation based on the simple Curie law.

The paramagnetic dipole moment obtained from the susceptibility for one mole of a free radical with one (and only one) unpaired electron is always the same, namely, $\sqrt{3} = 1.73$ Bohr magnetons. This corresponds, according to Curie's law, to a paramagnetic molar susceptibility, listed in table II, as a function of temperature. If in some compound, suspected to be a free radical or to contain one, the molar susceptibility at a given temperature is found to be smaller than indicated in the table, *e. g.*, < 1257 at 296° K. (23° C.), then the ratio of the molar susceptibility found to 1257 is the ratio of the amount of free radical to the total amount of substance, which may be a diamagnetic, chemically related molecular species.

⁴⁴ W. Klemm, *Magnetochemie*. Akadem. Verlagsgesellschaft, Leipzig, 1936.

For the method of slow reduction, the above ratio indicates the ratio of the free semiquinone radical to the total amount of the dye including all its levels of oxidation-reduction and polymerization.

TABLE II
MOLAR SUSCEPTIBILITY, χ , IN C.G.S. UNITS, FOR A FREE RADICAL WITH ONE UNPAIRED ELECTRON

Temperature, T		$\chi \times 10^4$
Absolute, ° K.	Experimental, ° C.	
304	31	1223
300	27	1240
296	23	1257
292	19	1275
288	15	1290
283	10	1315
273	0	1362
223	-50	1670
173	-100	2160

8. Measurements at Low Temperatures

The dependence of paramagnetic susceptibility on temperature, discovered by P. Curie, is one of the fundamental facts on which the theory of magnetism is founded. In general, no magnetic study of a chemical compound may be said to be complete if the susceptibility is measured only at one temperature. Measurements at very high temperatures will be important only for ferromagnetic substances and will not be of much interest in organic chemistry. Comparative measurements of susceptibility at temperatures ranging from room temperature down to that of liquid air are necessary for paramagnetic substances in order to ascertain the value of the constant, ϑ , in equation (10). As mentioned above, all measurements on free radicals⁴⁵ have shown that, in comparison with T , ϑ is very small if T is about room temperature. Diamagnetism is independent of temperature. It is, therefore, understandable that measurements at very low temperature have not often been essential for the clarification of structural problems in organic chemistry. However, there is one type of experiment in which a change in temperature is significant, namely, where an equilibrium exists in solution between a free radical and its parent substance, as for the radicals formed by dissociation of a hexaaryl ethane. The equilibrium of this dissociation shifts with the temperature. For measurements of susceptibilities at different, especially at very low, temperature, the methods must be modified accordingly.

The cylindrical vessel containing the specimen is suspended within a long, cylindrical, glass tube of somewhat wider diameter; and this glass

⁴⁵ L. Michaelis and M. P. Schubert, *Chem. Revs.*, **22**, 437 (1938).

tube is surrounded by a properly shaped Dewar flask containing the cooling liquid. The Dewar flask is as narrow as possible between the poles and is widened below and especially above the pole gap. A relatively simple device of this kind is reproduced in figure 15 from Klemm.⁴⁴ The glass tube surrounding the tube with the specimen should be thoroughly dried and fitted tightly into the opening in the floor of the balance case. The latter contains a drying agent. Although gradually there is some condensation on the tube, which shifts the taring weight, this change is slow and can be taken into account.

The following cooling liquids may be used: liquid oxygen (to -180°C.); alcohol-carbon dioxide mixture (to -78°C.). For measurements from -80° to -180°C. , a block of aluminum, containing cylindrical borings of various diameters, is first put in the Dewar vessel and cooled down with liquid air to the lowest temperature desired. Then the temperature of the aluminum block rises slowly enough to allow several measurements at various temperatures. The rise of temperature can be slowed down, if necessary, by leading a stream of precooled air into one of the borings. To avoid the condensation of dew, mentioned above, the tube may be filled with dry hydrogen or helium. Thermocouples are inserted for the measurement of temperature.

Many more elaborate modifications of this procedure have been used which afford high precision. Of these, the apparatus devised by Freed and Sugarman⁴⁶ may be shown in detail (Fig. 16).

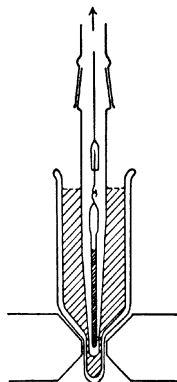


Fig. 15.—Arrangement for measurement at low temperature.⁴⁴

Glass tube, *G*, is suspended in an atmosphere of stagnant hydrogen, inside a tube, *H*, which is kept at low temperature by the surrounding refrigerating liquid. A stream of dry hydrogen enters the stationary tube, *H*, at the upper end, *J*, and flows to the outside atmosphere at such a rate as to keep the air with its moisture from descending during the experiment and affecting the weight of tube *G*. Baffles with small openings are mounted horizontally on the ends of tube *K*, which is perforated to allow the easy entry of hydrogen from tube *J*. The refrigerant is kept in the Dewar vessel, *F*. Its cap is provided with connections to a manometer and a pump, *P*, for reducing the pressure and consequently the temperature of the refrigerant. Tube *T-T* contains single-junction thermocouples which abut into *H*. The temperatures at the different levels of the hydrogen atmosphere as read by the thermocouples rarely vary by more than one-half degree. *B* is a capillary tube for the passage of air into the refrigerating liquid to lessen the bumping of the liquid

⁴⁶ S. Freed and N. Sugarman, *J. Chem. Phys.*, 11, 354 (1943).

while it is being pumped. Dewar vessel *F* has a circular cross section except at region *C*, which fits into the pole gap and is designed to separate the pole pieces, *M*, as little as possible and yet accommodate tubes *T* and *B*.

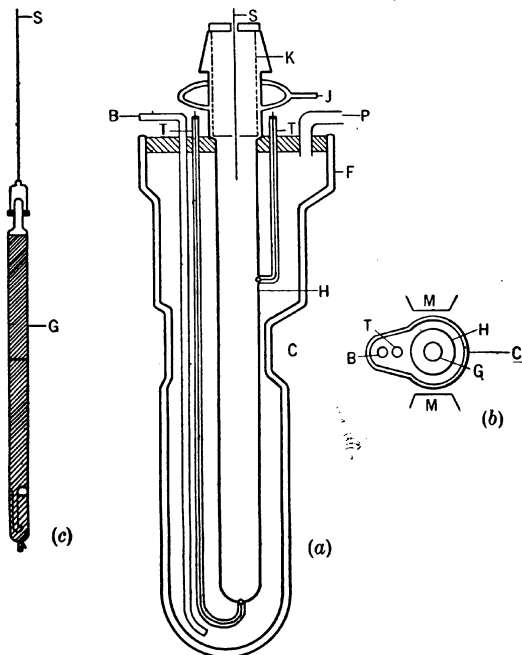


Fig. 16.—Apparatus for measurement at low temperature (Freed and Sugarman).⁴⁶

Recently, Calvin and Barkelew⁴⁷ published another device for measurements at low temperatures, constant to within $\pm 0.5^\circ \text{C}$. The sample is suspended inside a cylindrical vessel past which a stream of constant-temperature air is blown rapidly. The stream of air is produced by boiling liquid air, the temperature being controlled by the rate of boiling which is controlled by an electric heater. The current through the heater is regulated by a rheostat. The control is automatic; the thermocouple, near the pole gap of the electromagnet, controls a system of magnetic valves which operates the liquid air stream. A more detailed description of this apparently very efficient design would be welcome.

⁴⁷ M. Calvin and C. H. Barkelew, *J. Am. Chem. Soc.*, **68**, 2263 (1946).

9. Magnetic Anisotropy

The magnetic susceptibility of a particle may be different along different axes. Such a particle is said to be magnetically anisotropic. All methods described above measure only the average susceptibility because of the random distribution of either the crystalline powder or the dissolved molecules. The measurement of magnetic anisotropy has become a useful tool in the study of the crystal lattice,⁴⁸ especially in aromatic crystals. In the following, only the principles of the methods of measurement will be described. The fundamental discovery which makes clear the importance of magnetic anisotropy for the study of crystal structure⁴⁹ is that an aromatic ring system is magnetically anisotropic. Its diamagnetic susceptibility in a direction perpendicular to the plane of the ring is much greater than in the plane of the ring. A quantitative theory of this phenomenon has been developed by Pauling.⁵⁰ It had been known before that graphite exhibits this phenomenon even to a larger extent than do aromatic ring compounds. The magnetic anisotropy of an aromatic crystal results from the anisotropy of the aromatic ring, as well as its spacial orientation in the crystal lattice. The magnetic anisotropy of the molecule as such, the magnetic anisotropy of the crystal, and the configuration of the crystal lattice are so correlated that when two of these properties are known the third can be derived. Banerjee⁵¹ has made a comprehensive study of magnetic anisotropism of aromatic compounds in the crystalline state.

A method of studying the magnetic anisotropy of a crystal has been developed by Rabi.⁵² The crystal is suspended vertically in an inhomogeneous field (Figs. 17 and 18). The suspension is a glass thread thick enough to prevent turning of the crystal due to the magnetic force. This thread is attached to a graduated head making it possible to rotate the crystal. The crystal is attached, with proper regard to its orientation, to a very fine glass thread, 10 cm. long, by means of a minute amount of shellac. The thread, in turn, is attached to the rest of the suspension, which is arranged symmetrically between the pole pieces in the inhomogeneous part of the field. The orientation of the crystal is ascertained by sending a narrow beam of light along the *Y* axis and reflecting it from a known face of the

⁴⁸ K. S. Krishnan, *Trans. Roy. Soc. London*, **A231**, 235 (1933); **A232**, 99 (1933); **A234**, 267 (1935). K. Lonsdale and K. S. Krishnan, *ibid.*, **A156**, 597 (1936). W. A. Wooster, *Textbook of Crystal Physics*, Macmillan, New York, 1938.

⁴⁹ C. V. Raman and K. S. Krishnan, *Proc. Roy. Soc. London*, **113**, 511 (1927).

⁵⁰ L. Pauling, *J. Phys. Chem.*, **4**, 673 (1936). See also K. Lonsdale, *Science Progress*, **32**, 677 (1938).

⁵¹ S. Banerjee, *Z. Krist.*, **100**, 316 (1938).

⁵² I. I. Rabi, *Phys. Rev.*, **29**, 174 (1927).

crystal. This, together with the crystallographic data, makes known the orientation of the crystal.

The crystal, 3–4 mm. thick, is suspended in a liquid in which it is insoluble, for instance, in a saturated solution of the substance, and the susceptibility of this liquid is adjusted by adding either manganous chloride, to make it more paramagnetic, or potassium iodide, to make it more diamagnetic, to such an extent as to make the susceptibility of the liquid equal to that of the crystal in the particular orientation. The attainment of such a compensation is checked as follows. A movement of the crystal along

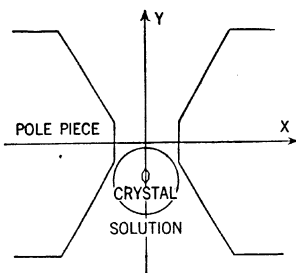


Fig. 17.—Placing of crystal as seen from above.⁶²

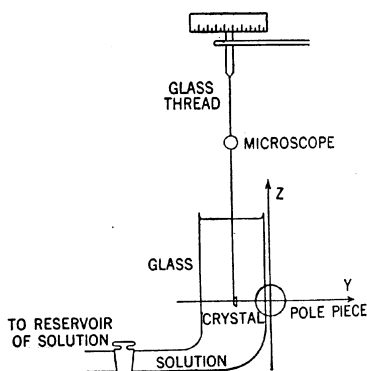


Fig. 18.—Side view of the crystal and suspension.⁶¹

the Y axis is observed by means of a microscope. Furthermore, the displacement of the crystal along the X axis is also observed. By means of the graduated head, the crystal is adjusted until there is no movement along the X axis when the field is turned on. Then the movement along the Y axis is observed. The composition of the liquid is adjusted so that no movement along the Y axis occurs. Then the volume susceptibility of the liquid equals that of the crystal along the Y axis. The susceptibility of the compensating liquid is measured with any of the methods previously described. The experiment is repeated for various orientations of the crystal.

Another method, which yields directly the difference between susceptibilities of a crystal along different axes instead of measuring the absolute values in each single direction, is described by Krishnan, Guha, and Baner-

jee.⁵³ This method yields only the difference between susceptibilities in different directions, but this with greater precision than by the other method. It is based on the following principle. As discussed before, two effects take place when a diamagnetic crystal is suspended by a quartz fiber in a magnetic field. There is an orientation, due to a force couple, and a displacement in the direction of decreasing field strength. The force couple is due to the asymmetry of the shape of the crystal and the inhomogeneity of the field, and to the magnetic anisotropy of the crystal. The couple due to the shape of the crystal may be eliminated by giving the material the form of a sphere. Then, only the couple due to the anisotropy remains. However, it can be shown that the couple due to the nonspherical shape is negligibly small within a *homogeneous* field. Therefore, the crystal is suspended in an homogeneous field and the force couple due to the anisotropy of the unit cell is deduced from the period of oscillation of the suspended crystal. We suppose that the crystal is suspended in an homogeneous field of strength, H , and that χ and χ' are the maximum and minimum values, respectively, of the molar susceptibilities in the plane of oscillation. The χ axis will tend to place itself along the field. Let the torsion head be rotated so that the torsion in the field is zero. If now the crystal is allowed to execute torsional oscillation about this position, and if T and T' are the periods of oscillation when the field is on and when it is removed, then:

$$\chi - \chi' = \frac{T'^2 - T^2}{T^2} \frac{c}{H^2} \frac{M}{m} \quad (11)$$

where c is the torsional constant of the fiber, m the mass of the crystal, and M its molecular weight. All quantities on the right-hand side being known, one may obtain $\chi - \chi'$.

A detailed description of an apparatus based on the principle of this method is given by Rogers,⁵⁴ from which the following is quoted: "The crystal was attached to a fine glass thread about one inch long by means of a minute amount of glyptal; when necessary this was done in an optical goniometer so that the orientation with respect to the fiber axis could be accurately determined. The glass thread was in turn attached to the lower end of a fine quartz fiber ($3\text{--}7\ \mu$ in diameter) about one meter long.⁵⁵ The upper end of the fiber is attached to the pin of a torsion head (A, Fig. 19). The torsion head consisted of a ball bearing (B), the outer part of which was fixed, while the central part (C) had a 360° protractor attached to it

⁵³ K. S. Krishnan, B. C. Guha, and S. Banerjee, *Phil. Trans. Roy. Soc. London*, A231, 235 (1932).

⁵⁴ M. T. Rogers, *J. Am. Chem. Soc.*, 69, 1506 (1947).

⁵⁵ For the making of quartz fibers, compare J. Strong, *Procedures in Experimental Physics*. Prentice-Hall, New York, 1938.

above, and the quartz fiber below. For convenience in handling and centering, the pin to which the fiber was attached was set in a brass disk (*D* in Fig. 19) which was held to the bearing only by a thin layer of wax. The crystal was suspended centrally

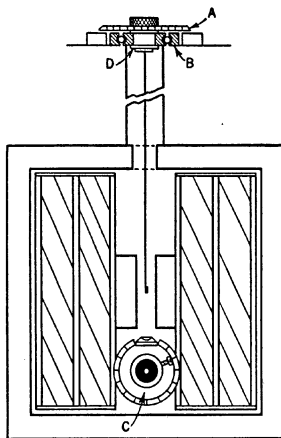


Fig. 19.—Roger's apparatus for the measurement of magnetic anisotropy.⁶⁴

between the plane parallel pole pieces (3 inches in diameter) of an electromagnet and observed from below. The viewing device (Fig. 20) enables the angle of rotation of the crystal in the field to be observed directly. An objective lens (*O* in Fig. 20) is focused on the illuminated crystal which is then observed in an eye piece (*E*)

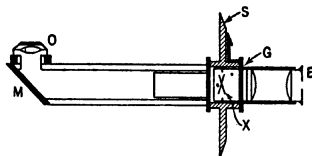


Fig. 20.—Viewing device of Roger's apparatus.⁶⁴

the mirror (*M*) being set at exactly 45° ; the rotation of the crystal is followed by reference to the crossed hairs (*X*), the angle of rotation being read directly on the eye piece scale (*S*). The crystal and fiber were protected from air currents by a glass case.

"The crystal was suspended in a known orientation and the torsion head adjusted initially so no motion of the crystal was observed on turning on the field.

The torsion head was now turned, with the field on, until the crystal had been rotated through 45° ; at this point the couple is a maximum and a further rotation of the fiber causes a sudden easily observed spinning of the crystal. From the angle (α) through which the torsion head was rotated, the torsion constant (K) of the fiber, the field strength (H), the mass (m) of the crystal, and its molecular weight (M), the anisotropy was calculated⁵⁶ by the equation:

$$\Delta\chi = \frac{MK}{mH^2} (\alpha_c - \pi/4)$$

where $\Delta\chi$ is the anisotropy (in c.g.s.u. per mole) in the plane perpendicular to the axis of suspension. The torsion constant of the fiber was obtained from the period of oscillation of an object of known moment of inertia; the field strength was measured (by this author) with a fluxmeter; small crystals were weighed on a microbalance. With monoclinic crystals a direct measurement was made of the angle, ψ , between the a axis and χ_1 , the axis of greatest magnetic susceptibility in the basal plane; this defines the orientation of the magnetic ellipsoid with respect to the crystal axes. The anisotropy, $\Delta\chi$, was determined for each crystal in three orientations. These values, along with the average susceptibility, enable the principal crystal susceptibilities to be calculated. The tensor algebra has been developed in detail by Lonsdale and Krishnan,⁵⁷ and Wooster.⁵⁸ Since only two measurements of $\Delta\chi$ are required, the third serves as an independent check on the values. Measurements of $\Delta\chi$ were made at field strengths of 6000 or 8375 gauss (occasionally at 4600 gauss) and the values so obtained averaged.

"The largest error in the measurements results from the couple exerted on an irregularly shaped crystal in the inhomogeneous component of the field. Where the shape anisotropy is large, as with a long needle, this error is important unless the field is quite homogeneous."

Since this method yields only the differences between susceptibilities along different axes, it is also worth while to describe a method, published by Krishnan and Banerjee,⁵⁹ which permits the measurement of at least the absolute value of the susceptibility in the direction in which it has its maximum, with a single crystal of 0.1 g. or less. The principle is similar to that in Rabi's method. Two aqueous solutions are prepared, one more diamagnetic, the other more paramagnetic, than the crystal, both of the same density as the crystal. A suitable mixture of these is kept in a thin-walled tube of 2-mm. bore, and a few well-developed crystals are dispersed in the liquid. The tube is placed in the strongly inhomogeneous part of the field. It is adjusted so that one of the crystals is in a position slightly apart from the central axis of the field, equidistant from the pole

⁵⁶ K. Lonsdale, *Reports on Progress in Physics*, **4**, 368 (1937).

⁵⁷ K. Lonsdale and K. S. Krishnan, *Proc. Roy. Soc. London*, **A156**, 597 (1936).

⁵⁸ W. A. Wooster, *Textbook of Crystal Physics*. Macmillan, New York, 1938.

⁵⁹ K. S. Krishnan and S. Banerjee, *Current Sci.*, **3**, 548 (1935).

pieces. The crystal is watched through a low-power microscope. It will turn around on switching on the field so as to place its axis of maximum susceptibility along the field, and, in addition, will be displaced. The mixture is then adjusted so that no displacement occurs. Then the susceptibility, per unit of volume, is the same as that of the mixture. The susceptibility of the latter is measured as usual. In contrast to the methods previously described, which yield the mean susceptibility, this method gives the susceptibility along that axis where it has its maximum. In combination with the method described for measuring differences along various axes, one obtains absolute values for the various axes. The difference between this procedure and that of Rabi is that in Rabi's method the crystal is suspended and free to rotate only in the plane perpendicular to the suspension, whereas in Krishnan's method the crystal floats and is free to rotate in space.

10. Organic Substances with Elements of the Transitional Group

Examples of the compounds to be discussed here are the complex compounds of organic substances with iron, nickel, cobalt, or copper in the cupric state. Among these are the biologically important iron-porphyrin compounds such as hemoglobin and the hemochromogens studied by Pauling and Coryell.⁶⁰ With respect to technique of measurement, nothing can be added. However, the evaluation of the permanent dipole moment from the observed susceptibilities of the paramagnetic compounds is more involved. The paramagnetic susceptibility of such metal complexes is largely due to the presence of one or more unpaired electrons on the central metal atom of the complex. If there are several of such unpaired electrons in one atom, they have "parallel spins." If it were true that in such compounds the permanent dipole moment depends only on the spin of electrons as in free organic radicals, quantum mechanics would enable one to correlate the magnetic dipole moment with the number, n , of unpaired electrons in the metal atom, according to equation (7) and the paramagnetic susceptibility per gram atom of the metal atom would be simply related to the moment by equation (10). Thus, from the susceptibilities one could calculate the moment and from it the number of unpaired electrons. Then, according to the theory which has been mainly developed by Pauling,⁶¹ inferences can be drawn regarding the nature of the bonds, covalent or ionic, and the spacial configuration of the complex, for instance, whether it is octahedral or tetrahedral or square. However, the following complications arise:

(1) It is not true that the magnetic effect of the orbital contributions

⁶⁰ L. Pauling and C. D. Coryell, *Proc. Natl. Acad. Sci. U. S.*, **22**, 210 (1936).

⁶¹ L. Pauling, *The Nature of the Chemical Bond*. Cornell Univ. Press, 1939, p. 9.

of the electrons is entirely quenched. The quenching is due to the facts that the motion of the electrons takes place in an electrostatic field produced by the electrons and nuclei in the environment and that this field can never be spherically symmetric. This field, because of its asymmetry, restrains the orientation of the orbitals and prevents them from being influenced by the applied magnetic field. (It may be recalled here that the magnetic properties due to the *spin* of the electrons are not influenced by the molecular electric field.) However, this restraining effect is not complete. For the rare earth metals, the orbital contributions are in fact of the same importance as the spin contributions because the shell with the unpaired electrons is surrounded by a complete electron shell which shields the effect of the surrounding molecular electrostatic field and allows the orbitals to orient themselves freely in a magnetic field. For the atoms of the iron group, the magnetic contributions of the orbitals are quenched, but not entirely. If the outer shell of such an atom is complete, or if one of its subshells contains accurately half the number of a complete subshell, as in the ferric ion, where the $3d$ subshell contains five electrons (ten would make up a complete $3d$ subshell), the vectorial sum of the orbital contributions is zero and orbital contributions need not be considered. In all other cases, there is left a small, not very well predictable, orbital contribution, which may amount up to one-half Bohr magneton or even somewhat more. For instance, in potassium ferriocyanide, which is considered to be an octahedral complex with covalent bonds and one odd electron, the effective moment, $\mu_{\text{eff.}}$, is found to be 2.3 B.M., instead of 1.73. On the other hand, two unpaired electrons would produce by their spins alone a moment of 2.8 B.M. Since the orbital contribution can only increase the moment and the observed moment happens to be smaller than 2.8, the best interpretation as to the number of unpaired electrons is that there is just one, and the difference, $2.3 - 1.73$, represents orbital contribution.

(2) A second problem is whether, in such molecular species in which the paramagnetic atoms are very closely packed, the mutual *magnetic* interaction of the magnetic atoms may produce an effect similar to that of the applied magnetic field. The consequence, for paramagnetic substances (in contrast to ferromagnetic ones), would be that the observed susceptibility is smaller than would correspond to the spin and orbital contributions. It is possible that very small effects of this kind may sometimes occur in solids with densely packed paramagnetic atoms, but no case has become known in which such an effect of any appreciable magnitude, important for the structural interpretation of an organic compound, is manifested beyond doubt.⁶²

⁶² L. Michaelis and S. Granick, *J. Am. Chem. Soc.*, **65**, 1747 (1943).

IV. FERROMAGNETISM

A few words on ferromagnetism may be helpful. It is of no direct concern to the organic chemist except that occasionally a trace of a ferromagnetic impurity may falsify measurements on powders. The most common ferromagnetic impurity is the ferromagnetic modification of Fe_2O_3 .

Ferromagnetism can be recognized by the fact that the molar susceptibility varies with the field strength, while in the preceding section of this chapter molar susceptibility was always considered as independent of the field strength, provided the field strength is within the limits of the ordinary laboratory instruments. It is true that, in principle, there is also a "saturation effect" with increasing field strength in para- and diamagnetic substances. However, fields of sufficient intensity to show this effect can by no means be attained with even what may be called a strong electromagnet of ordinary type at temperatures not very close to absolute zero. In ferromagnetic substances, or in samples of any substance contaminated with ferromagnetic substances, susceptibility will decrease with increasing field strength, even in the range of low field strengths. If there be any suspicion of ferromagnetic contamination of a substance to be investigated, it is necessary to ascertain that the susceptibility is independent of field strength by repeating the measurement with different amperages under otherwise equal conditions. If there be a *slight* dependence of susceptibility on the field strength, the value extrapolated to extremely large field strength should be taken, for ferromagnetic susceptibility reaches a saturation value with increasing field strength. The relative contribution of ferromagnetism to the total susceptibility is, therefore, the smaller the higher the field strength. Provided the slightly ferromagnetic behavior of the substance is really due to a very slight contamination with a ferromagnetic substance, the above procedure may be used to minimize the effect of the contamination unless the latter can be removed.

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Determination of RADIOACTIVITY

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I. INTRODUCTION

Under radioactivity measurements are usually grouped the measurement of α -rays, β -rays, and γ radiation. All of these radiations are similar in their ability to produce ions in, and therefore render conducting, air and other gases, and also to produce a latent image in the photographic film so that it blackens on development. In this chapter, emphasis will be laid on that most commonly encountered problem in radiochemistry, and particularly in the use of radioactive isotopes as labeled elements in tracer studies, the measurement of β -ray activities of an unknown in terms of a standard. The measurement of α -ray and γ -ray activities will be more briefly considered.

In view of recent increases in the activity of the radioactive materials with which the chemist may be concerned, and therefore the growing danger of injury to the human personnel concerned with their manipulation, attention will also be paid to measurement of the radiation dosage to which the experimenter may be exposed. Because of the vast amount of radioactivity produced in controlled nuclear fission, chemists, both organic and inorganic, are confronted with increasing frequency with the desirability or necessity of making radioactive assays or measurements. The release of radioactivity presents very great potential hazards both to personnel intimately involved and to the general population. Investigations and research undertaken to develop means of minimizing this hazard often involve the organic chemist.

In the treatment of disease, artificially as well as naturally radioactive isotopes are already quite extensively used. All present evidence indi-

icates that the effects of all radiations from radioactive elements, as far as an individual tissue is concerned, are purely destructive. Radioactive materials are only of therapeutic use if destruction of a certain tissue is desirable and if, either through differential tissue sensitivity or through localization of the radioactive isotope, such preferential destruction can be obtained. Iodine administered as iodide to mammals localizes preferentially in the thyroid. For this reason radioactive iodine has been used successfully in the treatment of thyroid disease where it is desirable to depress thyroid activity by a partial destruction of the thyroid tissue. Also, it occasionally happens in metastatic cancer originating in the thyroid gland, that the tumor tissue retains the ability of the tissue from which it was derived of concentrating iodine. Such tumors have been effectively treated, and apparently destroyed, by administration of suitable amounts of radioactive iodine. The remaining thyroid, if any, is also destroyed by the high dosage necessary, but adequate substitution therapy techniques are known and can be used. Unfortunately, these thyroid tumors are the only malignant tissues known that possess the ability to concentrate several times the amount of particular inorganic elements as vital normal tissues.

On the other hand, it is likely that organic chemical entities exist which will be concentrated specifically by tumors derived from other body tissues and organs. If such chemical entities can be discovered and subsequently prepared incorporating a suitable radioactive isotope, they will represent exceedingly useful additions to our armory of weapons against cancer and perhaps other diseases. In research in this field under way or in prospect the organic chemists, synthetic and analytical, will make key contributions.

The use of distinctive isotopes also provides a tool useful or indispensable in the solution of various problems of organic chemistry and the closely related field of biochemistry. Essentially a label is provided by which a desired quantity of an element may be followed, either in the relatively simple environment of the chemistry laboratory or the much more complex environment of the living organism. By proper synthesis of the distinctive isotope into a more complex chemical entity, this entity is in turn tagged as long as the bond between the label and labeled substance is not broken.

Advantage can be taken in many ways of this ability to tag selectively a portion of the same element. The rates of reaction in a state of dynamic equilibrium may be determined by supplying one component distinctively tagged, and later isolating and measuring the amount of and tagged element now present in other components of the mixture. Not only may one often determine whether one chemical entity is the precursor of another, but, by suitable degradation of the end product, decide exactly what struc-

tural elements of the end product are derived from the labeled group initially supplied.

If chemical analysis is difficult or impractical, due perhaps to concentrations below those detectable by ordinary means, a radioactive isotope can sometimes be used in the reaction under study, and, because of the sensitivity of radioactive detection methods, the analysis then carried out with relative ease. If analysis is inaccurate due to difficulty in isolating, quantitatively in a pure state, the substance whose analysis is desired, the method of isotopic dilution, discussed in greater detail in the chapter on Mass Spectrometry, may provide a solution to the problem. More detailed descriptions of this method have been given by Kamen¹ and Radin.²

II. PHYSICAL NATURE OF RADIATIONS FROM RADIOACTIVE SUBSTANCES

There are five types of radiation from radioactive elements for which the problems of measurement usually arise.

1. Alpha Rays

The α -ray, as it is emitted from the atomic nucleus, is the doubly-charged helium ion. It is most commonly found as a particle emitted from certain natural or artificially prepared elements with atomic numbers greater than that of lead. Although of high energy, several million electron volts, it is characterized by its low penetrating power (never exceeding a few centimeters of air), and by the consequent large number of ions produced per unit length of path in a gas.

2. Beta Rays

The term β -ray is commonly used to designate electrons, either positive or negative in charge, originating from the nucleus of an atom during radioactive disintegration. More loosely, all high-speed electrons are sometimes designated β -rays. Here we use it only to designate electrons of nuclear origin. The average penetrating power of β -rays from different isotopes may vary from a very small fraction of a millimeter of aluminum, the element commonly used to measure stopping power, up to several millimeters. Thus the experimental setup used for β measurements may be very dependent upon the β energies encountered in the problem under investigation.

Unlike α -rays, which, when they originate from a single isotope, have predominantly about the same energy and therefore the same penetrating

¹ M. D. Kamen, *Radioactive Tracers in Biology*. Academic Press, New York, 1947.

² N. S. Radin, *Nucleonics*, **1**, No. 2, 48 (Oct., 1947).

power, the average energy of β -rays originating from a single isotope is much less than the maximum energy usually given in reference tables. This ratio varies from one isotope to another but to an accuracy of about 20% is about 40% of the maximum value. For a compilation of more accurate values of this ratio where it is known, see Marinelli, Brinckerhoff, and Hine.³

Certain isotopes, for example, the 47 day half-life Fe^{59} , decay by alternative transitions, giving β -ray groups of different maximum energy. Known data for such isotopes have been summarized in the reference noted above.³ With positron emitters each β -ray is accompanied by two annihilation γ -rays of 0.51 m.e.v. due to the final destruction of the positron. The ordinary Geiger-Müller counter designed for β -ray measurements has about 0.5% of the sensitivity to annihilation radiation quanta that it has for β -rays.

3. Conversion Electrons

Gamma radiation originating in an atomic nucleus may transfer all of its energy to an orbital electron of that atom. Such ejected electrons are known as conversion electrons. In contrast to β -rays of nuclear origin, such conversion electrons originating from a single shell and from a single γ -ray all have the same energy. The portion of potential γ -rays thus transferring their energy to electrons depends upon the nuclear species and ranges as high as 100%. Since the removal of an orbital electron leaves a vacancy in the electron shell that is promptly filled by a transition from an outer shell, the production of conversion electrons is accompanied by characteristic x-ray production.

4. Gamma Rays

By γ -radiation one usually means the high-energy electromagnetic radiation emitted from the nucleus of the atom during the course of nuclear disintegration. Quantum energies of this radiation usually lie from 100,000 to several million electron volts. A nucleus may emit several γ -rays during disintegration but they are found in discrete energy groups, rather than showing a continuous range of energies. Since γ radiation is usually much more penetrating than α or β radiation, experimental problems usually center around absorbing and detecting as much of the radiation as possible, rather than any difficulty involved in getting the radiation into the measuring apparatus. The annihilation radiation of the positron, except

³ L. D. Marinelli, R. F. Brinckerhoff, and C. J. Hine, *Revs. Modern Phys.*, **19**, 25 (1947).

for its site or origin and quantum energy, is indistinguishable from the nuclear γ -ray.

5. Characteristic X-Rays

Accompanying the production of K conversion electrons or disintegration by K-electron capture by the nucleus, characteristic K x-radiation is emitted. In some instances, for example, the 4 year half-life Fe^{55} , this x-ray plus low-energy Auger electrons represent the only radiation produced. In contrast to γ -rays, the characteristic x-radiation from elements of medium and low atomic number is very easily absorbed.

6. Other Radiations

Often the characteristic x-ray quanta discussed in the preceding section, instead of being emitted, may be absorbed by an electron of an outer shell giving rise to a low-energy conversion or Auger electron. Beta rays, in a manner similar to the accelerated electrons in an x-ray tube, will produce x-rays during rapid negative acceleration. Such x-rays or *Bremsstrahlung* may occasionally constitute a health hazard for which additional shielding is necessary in handling high concentrations of isotopes that are primary emitters only of β -rays.

Table I gives information concerning radiations produced by an assortment of radioactive elements. More complete data are available from the Segrè isotope chart⁴ and other standard sources.⁵⁻⁷

III. APPARATUS. GENERAL DISCUSSION

1. Photographic Recording

Their ability to produce a latent image on photographic film may be used as a means of detecting and measuring each of these foregoing types of radiation. This detecting and measuring medium is often used as a means of measuring the external exposure of workers in this field and therefore is an aid in keeping such exposure within safe limits. It is also particularly useful in instances in which information is desired on two- or three-dimensional distribution of α - or β -ray active material in solid specimens—up to the present, usually in connection with problems encountered in metallurgy, mineralogy, and biology. Several chemical applications of

⁴ E. Segrè, *AECD 2111* (April, 1948).

⁵ G. T. Seaborg and I. Perlman, *Rev. Modern Phys.*, **20**, 585 (1948).

⁶ G. Friedlander and M. L. Perlman, *Chart of the Isotopes*, General Electric Co., Schenectady, 1948.

⁷ "1947 Summary of Nuclear Data," *Nucleonics*, **2**, No. 5 (Part 2), 82 (May, 1948).

this technique, not yet thoroughly explored, indicate that in the future it will have important applications in organic chemistry.

The photographic density pattern obtained on developing a film previously in intimate contact with the surface of a solid containing radioac-

TABLE I
DATA ON RADIATIONS FROM VARIOUS RADIOACTIVE ELEMENTS

Element	Half-life	Maximum β energy, m.e.v.	Other radiations
H ³	12 years	0.011	None
C ¹⁴	5×10^3 years	0.145	None
F ¹⁸	112 minutes	0.7	Annihilation radiation
Na ²⁴	14.8 hours	1.4	2 γ quanta per β
P ³²	14.3 days	1.71	None
S ³⁵	87.1 days	0.17	None
K ⁴²	12.4 hours	3.58 75% 2.07 25%	1 γ quanta per 4 β
Ca ⁴⁵	180 days	0.25	None
Fe ⁵⁵	4 years	None	K x-ray, Auger electrons
Fe ⁵⁹	44 days	0.26 50% 0.46 50%	1 γ quanta per β
Co ⁶⁰	5 years	0.31	2 γ quanta per β
Se ⁷⁵	127 days	None	K x-ray, conversion electrons, complex γ
As ⁷⁷	40 hours	0.8	None
Br ⁸²	36 hours	0.46	2 γ quanta per β
Sr ⁸⁹	55 days	1.5	None
I ¹³¹	8 days	0.60	3 γ quanta per β
Th ²³¹ (UX ₁)	24 days	0.19	Probably weak γ
Pa ²³⁴ (UX ₂)	1.14 minutes	2.32	Probably 2% γ

tive material is commonly known as a radioautograph. Work by Marinelli and associates⁸ concerning distribution of radioactive iodine in the thyroid furnishes an illustration of this technique. The thyroid tissue from animals or human subjects to which the radioiodine had been administered was fixed and sectioned by the usual histological technique. Sections were then placed in contact with sensitive photographic emulsions for periods of a few days, after which the plates were developed and fixed in the usual manner. By comparing this completed radioautograph with the stained tissue section, important information concerning the location of the labeled iodine in the thyroid was obtained.

The exact technique used in securing radioautographs will vary depending upon, among other factors, the intensity of radioactivity in the ma-

⁸ L. D. Marinelli, F. W. Foote, R. F. Hill, and A. F. Hoecker, *Am J Roentgenol. Radium Therapy*, **58**, 17 (1947).

terial being radioautographed, the nature and the penetrating power of the emitted radiation, and the preciseness with which it is desirable to establish the location of the radioactive material. For β radiation, the most sensitive photographic emulsions easily available are those commercial x-ray films designed for medical or industrial radiography without intensifying screens. If the resolution desired is not much better than one millimeter such a film, after exposure, can be removed and developed, and the distribution of radioactivity can then be determined by a simple visual comparison of the developed film and the original object or a photograph thereof.

During the radioautograph exposure the photographic emulsion must be kept in uniform close contact with the subject by some means of applying uniform pressure. Lack of such contact is the most common cause of unsatisfactory results. Also, care must be taken that the chemical nature of the subject is not such that a developable density is produced by this means. For isotopes emitting penetrating β -rays this difficulty may be avoided by interposing a sheet of thin varnished cellophane between the subject and the photographic emulsion. For isotopes emitting β -rays of low energy, such as C^{14} , nylon films or mica sheets a few microns thick may be satisfactory.

If a greater resolution of the details of distribution is desired, several modifications of the above technique are necessary. (1) An upper limit to resolving power is set by grain size in the developed photographic emulsion; these are large in commercial x-ray film. Medium lantern slides are a great improvement in this respect, but at the cost of perhaps tenfold greater exposure necessary due to the lower sensitivity of this film. (2) If the photographic image is separated from the subject, and the two examined separately, establishing a 1:1 correspondence between the subject and photographic image becomes difficult at relatively high-power magnification. To overcome this second difficulty Bélanger and Leblond⁹ developed a method in which the photographic emulsion from a lantern slide is removed and spread over a tissue section. After radiation exposure the autograph is developed, fixed, and washed. The tissue sections are then stained, cleared, and the entire preparation mounted in balsam.

In a method first described by Evans¹⁰ the section containing radioactive material is mounted directly on the photographic emulsion in the dark room. After suitable exposure the plate is developed and the tissue stained *in situ*. Since the tissue and photographic image are in slightly different planes, they can be examined separately by alternately focusing the microscope on each image plane. The limit to which detail can be resolved by the above method probably does not exceed 100 μ , at least with a medium-

⁹ L. F. Bélanger and C. P. Leblond, *Endocrinology*, **39**, 8 (1946).

¹⁰ T. C. Evans, *Proc. Exptl. Biol. Med.*, **64**, 313 (1947).

energy β -ray such as that of I^{131} . At least in biological work, much greater resolution would be very useful. Biochemists carrying out tracer experiments would like to be able to localize the segregation of radioactively labeled materials into individual cells and even in morphologically different regions of a cell. Research is under way in several laboratories on this subject, and detailed discussions of currently used techniques have been published.¹¹⁻¹⁴

The use of C^{14} and H^3 as radioactive labels is particularly important because of the almost universal occurrence of these elements in materials with which the organic chemist and biochemist are concerned. Fortunately, from the standpoint of high resolution in radioautographs, both these elements have β -rays of low penetrating power; that of H^3 with a maximum energy of 0.015 is particularly favorable from this viewpoint.

Other improvements can be made by decreasing the thickness of the section studied and particularly by decreasing the thickness and grain size of the photographic emulsion, as well as by eliminating as far as possible background due to negative fog. Figure 1 shows a radioautograph of rat thyroid following administration of I^{131} , prepared by George Boyd, and using an experimental thin coating of the NTB emulsion developed by *Eastman Kodak Company*.

Unfortunately, every increase in resolving power seems to be accompanied by a corresponding increase in the amount of radioactive material necessary to produce the radioautograph. In seeking higher resolving power, it seems increasingly likely that otherwise satisfactory techniques may have limited application in biochemistry due to the likelihood that the radioactive intensities necessary will substantially affect normal metabolism. The usefulness of electromagnetic focusing suggested by Marton and Abelson¹⁵ seems particularly limited in biological work for this reason.

With α particle emitting elements it is more easily possible to obtain detail resolution of the order of cellular dimensions. In preparations similar to those of Evans,¹⁰ Endicott and Yagoda¹⁶ have demonstrated that, with polonium, individual α tracks may be followed to their origin with such accuracy that it can be determined whether they came from the cytoplasm or the cell nucleus. Eastman alpha-particle plates were used in these experiments.

Alpha-emitting isotopes are limited to the heavier elements. Here too,

¹¹ A. M. MacDonald, J. Cobb, and A. K. Solomon, *Science*, **107**, 550 (1948).

¹² G. Boyd, *J. Biol. Phot. Assoc.*, **16**, 65 (1947).

¹³ T. C. Evans, *Nucleonics*, **2**, No. 3, 52 (March, 1948).

¹⁴ A. Gorbman, *Nucleonics*, **2**, No. 6, 30 (June, 1948).

¹⁵ L. Marton and P. H. Abelson, *Science*, **106**, 69 (1947).

¹⁶ K. M. Endicott and H. Yagoda, *Proc. Exptl. Biol. Med.*, **64**, 170 (1947).

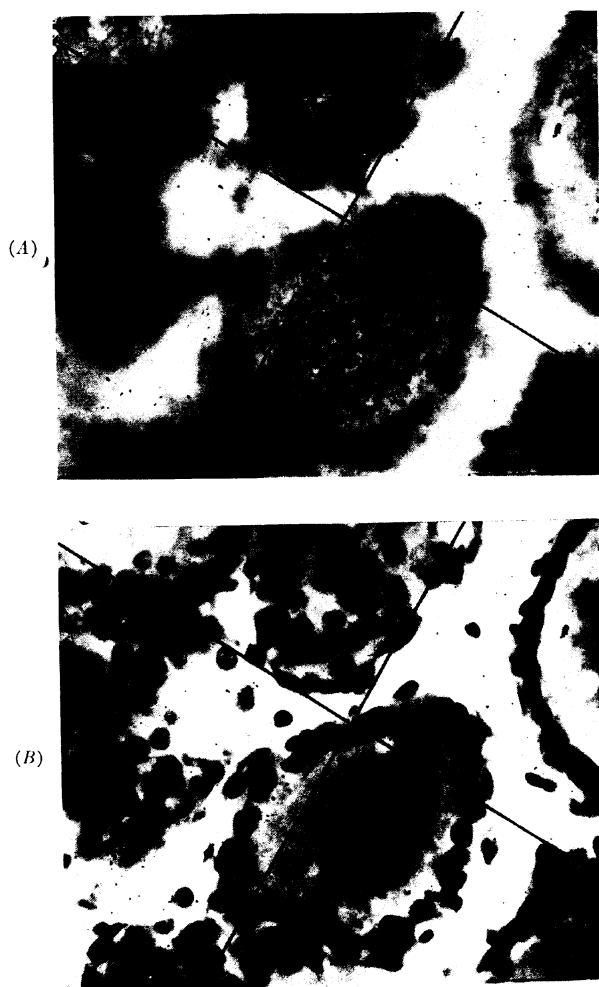


Fig. 1.—Radioautograph of rat thyroid following administration of I^{131} : (A) photomicrograph at emulsion level showing radioiodine distribution as indicated by developed silver granules; (B) photomicrograph of same field at tissue level

ultimate resolution in biological systems is limited by the destructive power of the radiations. The radiation dose of an α particle passing through a cell nucleus is of the order of 100 roentgens equivalent physical. Radiation-sensitive cells are probably often rendered abnormal or killed by one α -particle passing through the nucleus.

FILTER PAPER PARTITION CHROMATOGRAPHY

Gordon, Martin, and Synge¹⁷ and Consden, Gordon, and Martin¹⁸ originally developed filter paper partition chromatography as a means of detection and semiquantitative determination of amino acids. Other investigations have demonstrated the applicability of this technique to other types of low molecular weight organic compounds such as reducing sugars, purines, and organic acids. The range and sensitivity of this technique may be greatly extended by the use of radioactive reagents plus the preparation of radioautographs of the resulting filter paper chromatograms, as shown by preliminary work.¹⁹

An important development in the use of this technique will certainly be as a means of following the fate of chemical entities labeled by radioactivity in chemical and biological systems. It is particularly useful in that it makes possible the isolation and identification of breakdown or synthetic derivations of the original labeled substance without the necessity of a prior knowledge or even a good guess as to the precise nature of these substances.^{20, 21} Thus, a tool is available that may be expected to demonstrate the existence of intermediaries in biological metabolism, as well as in chemical reactions, whose existence is entirely unsuspected.

Since the resolution of fine detail will be unimportant in such chromatograms, the most sensitive photographic film should be used in the preparation of such radioautographs. The no-screen x-ray films seem well suited to this purpose. Photographic film is rendered developable by exposure to many chemical agents. Care must therefore be taken that such chemical fogging does not in fact occur by placing some inert and nonpermeable film, such as varnished cellophane, between filter paper and film during exposure or by running suitable control exposures with nonradioactive reagents.

The photographic method is probably at best semiquantitative. For quantitative results the radioactivity of selected areas of the chromatogram

¹⁷ A. H. Gordon, A. J. P. Martin, and R. L. M. Synge, *Biochem. J.*, **38**, 224 (1944).

¹⁸ R. Consden, A. H. Gordon, and A. J. P. Martin, *Biochem. J.*, **38**, 244 (1944).

¹⁹ A. S. Keston, S. Udenfriend, and M. Levy, *Federation Proc.*, **7**, 164 (1948).

²⁰ R. M. Fink, C. E. Dent, and K. Fink, *Nature*, **160**, 801 (1947).

²¹ R. M. Fink and K. Fink, *Science*, **107**, 253 (1948).

may be quantitatively measured by the use of a bell-type counter, a method used by Tomarelli and Florey in investigating the chemical form of radioactive sulfur excreted in the urine.²¹ Alternatively, the desired area of the filter paper may be cut out and its radioactivity determined after digestion or elution by an appropriate standard method.

2. Instruments for Ionization-Current Measurement

Except for health protection, for which accuracies of radiation measurement greater than 20% are not commonly sought, and the type of problem in which its property of spatial discrimination (as illustrated above) makes it valuable, the rather involved relationship between the intensity and total amount of radiation and the density of film blackening has limited the use of the photographic method. Instead, advantage is usually taken of the great sensitivity of methods based upon the electrical conductivity of air or other gases produced by the passage of α -particles or electrons. Rather complete bibliographies of detecting devices for ionizing radiations have been published.²²⁻²⁴ For convenience we may divide this type of measuring instrument into three main classes based upon the method used in determining the magnitude of the ionization current.

(1) *Electrometers and Electroscopes*.—The electrical charge carried by the ions is collected and produces a physical displacement of some part of the detecting apparatus that can be directly related to the magnitude of the ionization current.

(2) *Vacuum-Tube Electrometers*.—The ionization current is amplified by electrical means so that a relatively rugged current-measuring device can be used as an indicating or recording unit. Ordinarily, d.-c. amplifying circuits are used. An important exception is the class of instruments known as "dynamic condenser electrometers" which allow the use of a.-c. amplifying circuits.

(3) *Pulse Counters*.—Here the individual pulses of ionization current are so amplified that discrete counts of ionizing particles can be made.

A significant development of the past few years has been the increased availability on the commercial market of radioactivity detecting and measuring devices. A listing of sources for such apparatus is available from the Isotopes Branch of the Atomic Energy Commission at Oak Ridge, Tennessee.

²² M. Healea, *Nucleonics*, 1, No. 4, 68 (Dec., 1947).

²³ M. Healea, *Nucleonics*, 2, No. 2, 63 (Feb., 1948).

²⁴ M. Healea, *Nucleonics*, 2, No. 3, 66 (March, 1948).

3. Electrometers and Electroscopes

The electroscope differs from the electrometer in using no source of potential other than the one being measured. The electrometer uses one or more auxiliary potential sources.

As in the well-known gold leaf electroscope, the operation of these instruments depends on the tendency of charges of like sign to repel one another. In electroscopes the gold leaf is usually replaced either by quartz fibers onto which an electrically conducting coating of a noble metal has been deposited, usually by evaporation, or by exceedingly thin filaments of Wollaston wire.

A widely used and sensitive type of instrument for ionization current measurements is the Lauritsen electroscope.²⁵ Figure 2 shows schematically the essential parts of this instrument. In a manner similar to the familiar gold leaf electroscope, its operation depends on the tendency of electrical charges of the same sign to repel each other. The gold leaf is replaced by a quartz fiber rendered conducting by a thin deposit of a noble metal and is so light that the effect of gravity is small and the restoring force is that of the elasticity of the quartz fiber. The fiber is fastened by one end parallel to, and is electrically connected to, a metal bar that may be charged to any desired potential, usually of the order of 100 volts. This potential is provided by batteries or a charging unit, electrostatic in principle or powered from the house line. The repulsion of the fiber by the bar is a function of the charge. This deflection is read by observation with the microscope of a second, short, quartz segment fastened at a right angle to the tip of the free end of the quartz fiber. With the charged electroscope connected to the well-insulated electrode of an ionization chamber, the ionization current is read by determining the rate of loss of charge from the electroscope as determined by the changed position of the quartz fiber against a calibrated scale over measured periods of time. The ionization chamber may be a separate container connected to the electroscope, or more likely, when the greatest sensitivity is desired in the measurement of nongaseous samples, the chamber containing the sensitive element of the electroscope acts as the ionization chamber with the charged element of the electroscope acting as one electrode. Thus, electrical capacity is minimized and sensitivity kept at a maximum.

It is quite essential that this instrument be protected from drafts and temperature inequalities in a manner similar to that used in a microbalance, since convection currents can seriously deflect the light quartz fiber, invalidating measurements of radioactive samples of moderate activity.

²⁵ C. C. Lauritsen and T. Lauritsen, *Rev. Sci. Instruments*, **8**, 438 (1937).

For weaker samples the time of measurement, two or three hours for each sample and blank, tends to be inconveniently long.

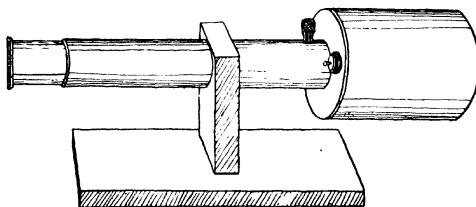


Fig. 2a.—Lauritsen electroscope.

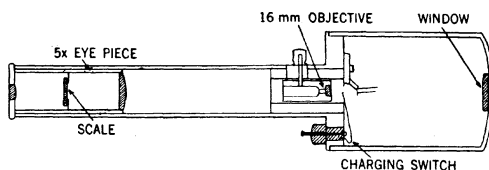


Fig. 2b.—Schematic drawing of Lauritsen electroscope.

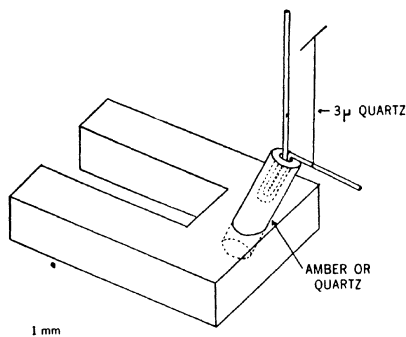


Fig. 2c.—Details of quartz fiber mounting.

An electroscope developed by Landsverk and Wollan²⁶ is closely related to the Lauritsen electroscope, but is somewhat more rugged and stands abuse better. It is widely used in portable instruments designed for health protection.

Regener's electroscope, a single-fiber type supported at both ends, is

²⁶ O. G. Landsverk, *Manhattan District Declassification Code 396* (Nov. 1, 1947).

used in the Victoreen r -meter. Neher²⁷ has described a torsion electroscope that probably has the highest useful sensitivity of any electroscope and approaches the sensitivity obtainable in electrometers. This same reference provides a very good general discussion of the various types of electroscopes and electrometers and their respective advantages and disadvantages.

For radioactive measurements, the most widely used electrometer is patterned after the one designed by Lindemann.²⁸ An improved design, developed at the University of Chicago Laboratories of the Manhattan Project, is now commercially available. It is a quadrant electrometer in which the deflection of a quartz needle is read through a microscope. Because it is not critical as to position, it is well adapted to semiportable use. As with other electrometers, an auxiliary ionization chamber must be used since the indicating mechanism is so shielded that there can be no efficient collecting of ions from a surrounding volume of gas. It has a useful sensitivity of about 500 divisions per volt, representing a charge sensitivity with no external capacity of about 5×10^{13} divisions per coulomb.

Of all electrometers, probably the best engineered for high sensitivity is the Hoffmann instrument.²⁹ According to Neher²⁷ a maximum sensitivity can be reached with this instrument of 5×10^{15} divisions per coulomb. Drift has been eliminated to such an extent that, by taking sufficient time, currents as low as one electron per second (1.59×10^{-19} amperes) can be detected. Before the war Hoffmann electrometers were available from *E. Leybold's Nachfolger A. G.*, Koln-Bayental, Germany. Their present availability is not known.

4. Thermionic Vacuum-Tube Electrometers

Advantage is often taken of the current- and voltage-amplifying abilities of thermionic vacuum tubes to amplify ionization currents to the point where they can be measured or recorded with rugged instruments. For an input tube to such a d.-c. amplifier, it is desirable that the control grid current be small compared with the ionization current; otherwise, fluctuations not easily avoided in this current will give rise to instability of amplifier output. Specialized thermionic vacuum tubes, known as electrometer tubes, have been especially developed for this purpose. The General Electric FP-54 has, for the past fifteen years, been used for this purpose in semipermanent setups, often employing circuits developed by DuBridge

²⁷ H. V. Neher, in J. Strong, *Procedures in Experimental Physics*. Prentice-Hall, New York, 1938, Chapter 7.

²⁸ F. A. Lindemann, A. F. Lindemann, and T. C. Keeley, *Phil. Mag.*, **47**, 577 (1924).

²⁹ G. Hoffmann, *Physik. Z.*, **13**, 480 (1912).

and Brown,³⁰ Turner,³¹ or Caldwell.³² In chapter 10 of Strong³³ details are given on the experimental use of these tubes. Applications of similar circuits to spectrophotometry are described in Chapter XXI of this book and to mass spectrometry in Chapter XXXI. A double triode of similar design, the Plotron 5674, has recently become available. Characteristics of a developmental model of this tube have been described by Lafferty and Kingdon.³⁴

Victoreen Manufacturing Company and *Raytheon Company* now manufacture miniature electrometer tubes with about a ten-ma. current drain which are well designed for portable use. A variety of portable instruments using these small tubes and designed primarily for personnel protection are now available commercially.

In a permanent installation, an electrometer tube can be made to give a voltage sensitivity without further amplification even higher than the Hoffmann electrometer. To obtain high sensitivity, the grid resistor is omitted and a floating grid method used. However, drifts in output difficult to avoid probably make their ultimate useful sensitivity somewhat lower than the Hoffmann electrometer. Their advantage lies in their great flexibility since the recording system can, if necessary, be located at a distance from the electrometer tube proper. Also, with additional amplification they can be made to give written records.

5. Dynamic Condenser Electrometers

High-gain amplifiers for a.-c. potentials can be designed with much greater stability than for d.-c. potentials. To obtain this advantage in practice, it is necessary to convert the d.-c. potential built up by ionization-current flow to an insulated electrode into an a.-c. type of signal. Various attempts to do so in the past have been used rotating variable condensers with commutators or slip rings. These sliding contacts have introduced so much contact "noise" that high sensitivity was not in fact obtained. It is only recently that two methods have been developed in which sliding electrical contacts are not used, and which have led to results appreciably better than those obtained with the FP-54 and a d.-c. amplifier as far as sensitivity and stability are concerned. The principle of the two methods is shown in figure 3. In figure 3a when switch *S* is opened, the system *A* will gradually rise in potential as a result of flow of ionization current in

³⁰ L. A. DuBridge and H. Brown, *Rev. Sci. Instruments*, **4**, 532 (1933).

³¹ L. A. Turner, *Rev. Sci. Instruments*, **4**, 665 (1933).

³² P. A. Caldwell, *Rev. Sci. Instruments*, **19**, 85 (1948).

³³ J. Strong, *Procedures in Experimental Physics*. Prentice-Hall, New York, 1938.

³⁴ J. M. Lafferty and K. H. Kingdon, *J. Applied Phys.*, **17**, 894 (1946).

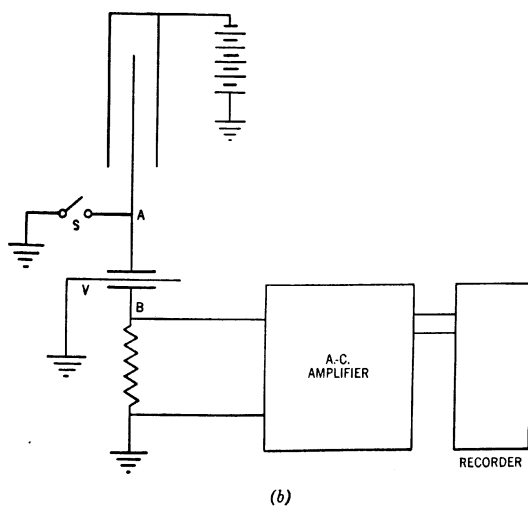
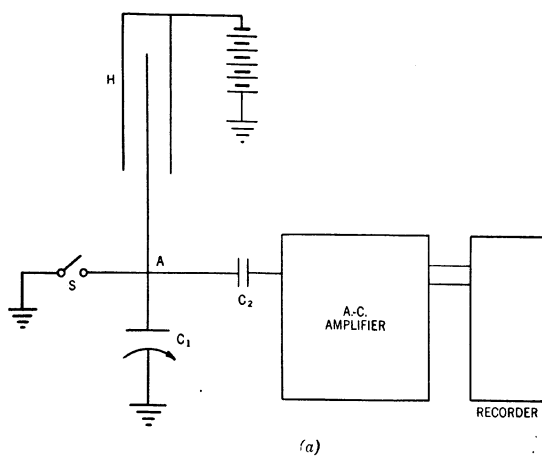


Fig. 3.—Dynamic condenser electrometer.

chamber H . A periodically changing variable condenser, C_1 , will change the electrical capacity of system A and, therefore, the signal voltage fed through condenser C_2 into the amplifier. When the potential of A is zero with respect to ground, there will be no signal. As the charge on A increases, the a.-c. signal fed into the amplifier will increase proportionally.

In figure 3b, the condenser plate connected to the charge-collecting system, A , is ordinarily shielded from plate B by a grounded metal plate, V . Provision is made for alternately inserting or removing this plate or vane from between the condenser plates. Thus, the input of the amplifier is alternately connected by capacitance coupling first to ground potential and then to the potential of the ion-collecting system. As this collection potential progressively increases from ground, an a.-c. signal of increasing magnitude is fed into the amplifier.

The method of figure 3a is utilized in the vibrating reed electrometer developed at the University of Chicago³⁵ and now available commercially. The variable condenser is a gold-coated iron reed which, magnetically driven, vibrates close to the other plate, thus providing a variable capacity to the system. The method of figure 3b is utilized in the dynamic vane electrometer (unpublished) developed at the University of Rochester.

The dynamic condenser electrometers can be made with useful voltage and charge sensitivities as great as any electrostatic instrument including the Hoffmann electrometer. If properly constructed, they possess very very good stability with little drift. A very great advantage is that they can operate in any position, are not especially sensitive to mechanical shocks, and thus do not need elaborate antishock mounting. By their nature they are easily made to give written records by pen and ink recording systems. The rather elaborate and careful machining necessary in their construction makes them expensive. Also, since they use electronic amplifiers, experienced servicing personnel are occasionally necessary.

6. Apparatus for Counting Ionizing Particles

A distinct type of detecting and measuring instrument is one designed not to measure total ionization produced in an ionization chamber by an ionizing source but rather to detect and record single pulses of ionization current produced by individual ionizing particles. An extensive discussion of these types of pulse counters has been given by Lewis,³⁶ Korff,³⁷ and Corson and Wilson.³⁸

³⁵ H. Palevsky, R. K. Swank, and R. Grenchids, *Rev. Sci. Instruments*, **18**, 298 (1947).

³⁶ W. B. Lewis, *Electrical Counting with Special Reference to Counting Alpha and Beta Particles*. Cambridge Univ. Press, London, 1947.

³⁷ S. A. Korff, *Electron and Nuclear Counters*. Van Nostrand, New York, 1946.

³⁸ D. R. Corson and R. R. Wilson, *Rev. Sci. Instruments*, **19**, 207 (1948).

A. PRIMARY IONIZATION-PULSE COUNTERS

The ionization current produced by a β - and γ -ray, and even an α -ray, is not large enough to measure or record easily without amplification. In principle, the simplest instrument for accomplishing this is the linear amplifier attached to an ionization chamber of low electrical capacity. Under these conditions the amplifier pulse output is proportional to, but many times greater than, the ionization current produced by a single particle entering the ionization chamber. Work on nuclear energy during the war led to extensive development of apparatus of this type about which information is available from the United States Department of Commerce in the Manhattan District Declassification Code series, abbreviated MDDC, or in the process of publication in technical journals, or in the forthcoming *National Nuclear Energy Series*. Because of the amplification required and the resulting constructional and operational difficulties, this type of instrument is not much used except to detect the relatively large ionization pulses produced by α -particles. Here it is used to discriminate between such pulses and the small ones produced by β - and γ -rays. Equipment of this type has been recently placed on the market.

Crystal Counters.—An exception is based upon the discovery that silver chloride crystals³⁹ and diamond slabs at room temperature⁴⁰ can, under proper conditions, allow rapid collection of electrons liberated by α -, β -, and γ -rays. Even high-energy β -rays are strongly absorbed by such solids and relatively large electrical pulses obtained. Applications of this technique to radioactivity measurement remain for the future.

B. PROPORTIONAL COUNTERS

Advantage is sometimes taken of the fact, first reported by Rutherford and Geiger,⁴¹ that, with a sufficiently high voltage between the two elements of a properly designed ionization chamber, the current flow due to an ionizing particle will be increased many times by secondary ionization produced by the impact of rapidly moving ions or electrons. Such a proportional counter usually consists of a fine (about five-mil) wire concentric in a metal tube provided with a thin window or port for sample introduction. Under proper conditions these output pulses may be kept proportional to, although many times greater than, the primary ionization current. This amplification, taking place in the ionization chamber itself, greatly reduces the demands on the auxiliary amplifier, and yet allows the

³⁹ P. J. Van Heerden, *The Crystal Counter, A New Instrument in Nuclear Physics*. Noord-Hollandsche, Amsterdam, 1945.

⁴⁰ D. E. Woolridge, A. J. Ahearn, and J. A. Burton, *Phys. Rev.*, **71**, 913 (1947).

⁴¹ E. Rutherford and H. Geiger, *Proc. Roy. Soc. London*, **A81**, 141 (1908).

possibility of discriminating between α and β particles. A comprehensive discussion of an early version of this type of apparatus is given by Brubaker and Pollard.⁴² Recent work by Simpson⁴³ has led to the development of very reliable proportional counters, both for high-accuracy α counts on prepared samples and so-called hand and personnel counters for measuring activity so that health hazards due to α -emitting materials can be minimized. A possibility also exists of using proportional counters for β and γ measurement with the resulting advantages of short resolving times and possibly the ability to discriminate partially between the relatively concentrated ionization due to low energy β -rays, such as are produced by C^{14} , and the less dense ionization due to cosmic radiation and contaminating hard β and γ emitters.

C. FLUORESCENT AND SECONDARY EMISSION COUNTERS

The electron multiplier in which amplification by secondary emission is utilized⁴⁴ may have eventual use in radioactivity measurements as a direct detector of high-speed electrons or α particles. Also, the electron multiplier can serve as a detector of the flashes of light emitted by Cerenkov radiation⁴⁵ or of the fluorescence produced by ionizing radiation in inorganic or organic fluorescing material.^{46, 47}

The latter device is known as a "scintillation counter." In the present state of development this type of counter appears to offer two points of superiority over radiation detecting devices. First, the efficiency for detecting and counting gamma rays may be quite high, 10% or more of the radiation incident on the crystal, when using a crystal of dense material such as sodium iodide activated with thallium. Second, extremely high counting rates may be achieved, since the rise time of the pulse from the electron multiplier is 10^{-6} second or less using organic crystals such as naphthalene or anthracene.

With the present available photomultiplier tubes, the 931-A and similar types, cooling as with Dry Ice or complicated coincidence methods of counting are necessary to reduce, to a satisfactory level, tube noise that otherwise gives an apparently high background counting rate. Improvements in phosphors and photomultiplying tubes may in the future lead to radiation detecting devices that will be extensively used.

⁴² G. Brubaker and E. Pollard, *Rev. Sci. Instruments*, **8**, 254 (1937).

⁴³ J. A. Simpson, Jr., *Rev. Sci. Instruments*, **18**, 884 (1947).

⁴⁴ J. S. Allen, *Rev. Sci. Instruments*, **18**, 739 (1947).

⁴⁵ R. H. Dicke, *Phys. Rev.*, **71**, 737 (1947).

⁴⁶ G. B. Collins and R. C. Hoyt, *Bull. Am. Phys. Soc.*, **23**, No. 2, 29 (1948).

⁴⁷ M. Deutsch, *Nucleonics*, **2**, No. 3, 58 (March, 1948).

D. GEIGER-MÜLLER COUNTERS

At the present time the most widely used type of apparatus for ionization-pulse counting is the Geiger-Müller counter (hereafter called the G-M counter). The G-M counter, in its traditional form, consists of a metal tube with a concentrically stretched fine wire, these two parts serving as the two electrodes. It is usually enclosed in a glass envelope so that it may be filled with a desirable gas at a pressure of a fraction of an atmosphere. The applied voltage is so high, usually between 800 and 1500 volts, that with even a single electron liberated in the sensitive portion of the tube, ionization by collision will increase the number of current-carrying ions in the gas until a large current flows. In so-called fast or self-quenching counters, usually made so by the inclusion of certain organic vapors, for example, methyl alcohol, the discharge is self-extinguishing. In other counters this current flow can be stopped only by lowering the voltage applied to the tube below the point at which the electrical discharge is self-maintaining. One thinks of the G-M tube then as a trigger tube in a state of unstable equilibrium ready to pass an electrical discharge as soon as an ionizing radiation produces one or more free electrons to initiate this event.

The auxiliary electrical apparatus to be used with the G-M counting tube consists of, first, a steady and consistent source of high voltage to operate the tube. Second, there will be necessary, in the case of G-M tubes that are not self-quenching, a means of rapidly lowering the applied voltage once a discharge has been initiated until it is extinguished and then raising the voltage once more to its initial value so that the tube is reset and waiting once more for another discharge or count. This device, ordinarily called a quenching circuit, will usually be electronic in nature with one or more radio tubes. Under some conditions a simple high resistance, of the order of 10^9 ohms for tubes not self-quenching, may serve this purpose sufficiently well. Third, it will be necessary to have some means of amplifying and recording each pulse of current in the counting tube.

The discovery⁴⁸ of the Geiger-Müller tube counter in 1928 represented a culmination of increasing knowledge concerning the application of secondary ionization in gases to ionizing-particle counting techniques of which the work of Rutherford and Geiger⁴¹ and the discovery of the point counter by Geiger⁴⁹ are important stepping stones. Since its discovery, the G-M counting tube has undergone many developments, particularly in the design and production of specialized forms fitting it to various types of radiation measurement problems. Some of these will be more fully discussed in the section on β - and γ -ray measurements.

⁴⁸ H. Geiger and W. Müller, *Physik. Z.*, **29**, 839 (1928).

⁴⁹ H. Geiger, *Physik. Z.*, **14**, 1129 (1913).

Design of satisfactory G-M counting tubes, as far as satisfactory operating characteristics are concerned, has centered around the following problem: After a discharge or count has occurred in a counting tube and the voltage lowered by the quenching circuit, it is necessary that all the charged ions and excited molecules return to the ground state, or at least that no ions or atoms be left in metastable states with a possibility of later producing free electrons once the counter voltage is again raised to the operating point. For, if this does occur, there will be later counter discharges not connected with the presence of ionizing radiation from outside. The degree of success in solving this problem determines over what voltage ranges the counter will remain stable, and also how short the quenching period may be made, and, therefore, how many counts are missed by the tube due to low resolving time. For more detailed discussions of G-M counter design and operation see Brown,⁵⁰ Montgomery and Montgomery,⁵¹ and Korff.³⁷

Voltage Supplies.—To have the most general usefulness, the voltage supply designed for a G-M counter tube should be easily variable from 600 to 2000 volts, have a meter or other method of measuring voltage applied to the counting tube, and have the output voltage stabilized so that, within the range of line voltages encountered, the output does not vary by more than 0.5%. Obviously, a voltage supply designed for one specific counting tube may not need this voltage range. On the other hand, some tubes designed for C¹⁴ measurement may require voltages of 5000 or more. The current drain need not exceed one milliampere. An excellent discussion of voltage supply problems is given by Strong.³³

Voltages of this magnitude are dangerous, particularly if the circuit incorporates filtering capacities of one microfarad or more. High-frequency voltage generators can reduce this hazard because of the low filtering capacity required.

Quenching Circuits.—The function of the quenching circuit, once a discharge count has been initiated in the G-M tube, is to drop the voltage applied to the tube below the threshold voltage at which such a discharge can be maintained and then, with the discharge extinguished, rapidly to return the voltage to its initial value in readiness for another count.

The original electronic circuit designed for this purpose by Neher and Harper⁵² is still widely used. Figure 4 shows the essential portions of this circuit. Normally *T1*, a high- μ tube, is biased just beyond the point at which no current flows. The full potential of the high voltage supply is, therefore, across *T1* and also across the G-M counting tube. When a discharge occurs in the G-M tube, positive ions are collected by the cylinder causing the grid to become positive, and current flows through *T1*. The resulting voltage drop across *R2* lowers the voltage across the

⁵⁰ S. C. Brown, *Nucleonics*, 2, No. 6, 10 (June, 1948).

⁵¹ C. G. Montgomery and D. D. Montgomery, *J. Franklin Inst.*, 231, 447 and 509 (1941).

⁵² H. V. Neher and W. W. Harper, *Phys. Rev.*, 49, 940 (1936).

G-M tube below that at which an electrical discharge can be maintained, and it is extinguished. As the passage of current removes the voltage drop across R_1 , T_1 ceases to pass current, the voltage across the G-M tube rises to its original value, and the cycle of events is ready to be repeated on the next count. The electrical pulse is transmitted to a recording circuit through condenser C_1 .

The above circuit requires that both electrodes of the G-M counter be insulated from ground, and that the amplifying tube stands the full counter voltage. Many glass-enveloped, receiving-type radio tubes are satisfactory in this respect. To prevent troublesome leakage at the tube base, either a ceramic-based tube and ceramic or polystyrene socket should be used, or the base removed entirely and the tube mounted in a ring of insulating material.

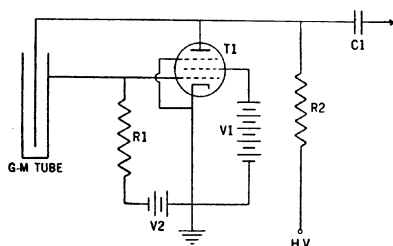


Fig. 4.—Neher-Harper quenching circuit.

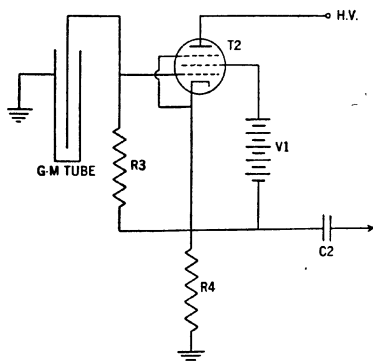


Fig. 5.—Neher-Pickering quenching circuit.

Another very widely used quenching circuit is that of Neher and Pickering,⁴² as shown in figure 5. Normally the grid and cathode are at about the same potential, the grid bias is nearly zero, and the voltage drop across the tube is small compared

⁴² H. V. Neher and W. W. Pickering, *Phys. Rev.*, **53**, 316 (1938).

with that across R_4 . When an ionizing particle enters the G-M tube, the grid of T_2 becomes negative because of the electrons collected by the central wire of the G-M tube, and the voltage across R_4 , and therefore across the counter, decreases until the discharge is extinguished. In this circuit, the quenching tube may be operated within its rated limits and no special preparation of the tube is needed. The counter cylinder operates at ground potential and so does not need to be insulated or separated from the sample being measured. This circuit requires a well-insulated filament supply and a high-voltage source capable of supplying a continuous current, usually about one milliampere.

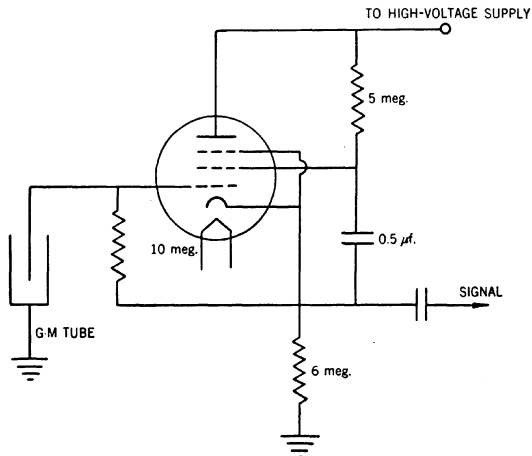


Fig. 6.—Neher-Pickering a-c. supply.

Adaptations of the basic Neher-Pickering circuit can be made to fit it for operation with an a-c. power supply without the use of accessory batteries. Such a circuit due to Kip, Bousquet, Evans, and Tuttle⁴⁴ is shown in figure 6. .

Many circuits already constructed and in use for G-M counting tubes have only a high resistance available for quenching. With many G-M tubes the performance obtained can be substantially improved by incorporating the following battery-operated version of the Neher-Pickering circuit as shown in figure 7. The filament current and screen grid voltages are furnished by portable radio *A* and *B* batteries that have a useful operating life of several months. Counts can easily be heard by connecting crystal earphones to the condenser output without further amplification. Care should be taken to insulate the battery and tube properly since they will operate at the potential of the G-M tube.

A combined voltage-regulating and -quenching circuit related to the Neher-

⁴⁴ A. Kip, A. Bousquet, R. D. Evans, and W. Tuttle, *Rev. Sci. Instruments*, 17, 323 (1946).

Pickering circuit is given by Bale and Bonner.⁵⁵ Getting⁵⁶ has described a third, quite distinct type of quenching circuit utilizing the principle of the multivibrator.

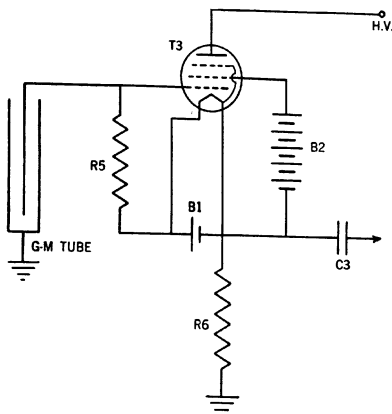


Fig. 7.—Practical Neher-Pickering circuit.

T3—1N5GT

B1—1.5-v. A battery

R5—5 megohms

B2—45-v. B battery

R6—15 megohms

C3—0.001 μ f.

Recording Circuits.—For quantitative work, since it is usual to have the electrical pulses from the G-M tube operate some type of mechanical counter or recorder, activity measurements are commonly made in terms of counts per minute. The simplest type of recording equipment has the mechanical counter register one count for every ionizing pulse. However, all mechanical counters register at a much slower rate than the G-M tube is able to count. Thus, the limit of radioactivity that may be measured is set by the mechanical recorder. It is, therefore, common practice to introduce an electronic scaling circuit such that the mechanical recorder registers every second, fourth, or eighth count, etc. A means of interpolation is provided so that the real number of counts, including the counts accumulated in the scaling circuit after the last registration on the mechanical recorder, can easily be computed.

Perhaps the most widely used circuit for this purpose was developed by Higinbotham, Gallagher, and Sands.⁵⁷ A decade counting circuit due to Potter⁵⁸ indicates counts in groups of ten, making reading of the final count somewhat more convenient. This is only one of several decade counting circuits recently developed.

⁵⁵ W. F. Bale and J. F. Bonner, Jr., *Rev. Sci. Instruments*, **14**, 222 (1943).

⁵⁶ I. A. Getting, *Phys. Rev.*, **53**, 103 (1938).

⁵⁷ W. A. Higinbotham, J. Gallagher, and M. Sands, *Rev. Sci. Instruments*, **18**, 706 (1947).

⁵⁸ J. T. Potter, *Electronics*, **17**, 110 (1944).

Rapid scaling circuits require an electrical pulse of uniform shape and size for reliable operation. To accommodate various types of pulses arising from G-M counting tubes or linear and proportional counters, scaling circuits are usually preceded by a pulse shaper or amplitude discrimination. Problems encountered in the design of scaling circuits as well as amplitude and pulse-shape discriminators are excellently discussed by Lewis.³⁸

Often it is desirable to take data in terms of the time required for a specified number of counts rather than the more conventional counting rate in counts per minute. Peacock and Good³⁹ have designed apparatus that gives a written record of the time required to accumulate a desired number of counts. Apparatus that automatically stops a timing clock at the moment of accumulation of a desired number of counts is commercially available.

Another means of recording high counting rates involves the use of a counting rate meter circuit. Here a meter or recording device indicates the average counting rate, usually in counts per minute. This method of quantitatively indicating activity is the one usually employed in portable meters designed primarily for health protection. Reliable apparatus of this type suitable for quantitative measurements have also been designed⁵⁴ using a recording milliammeter to give a written record.

A wide variety of G-M counting tubes and associated circuits are available from commercial sources.

IV. GENERAL PRINCIPLES OF BETA-RAY MEASUREMENTS

Most of the artificially radioactive elements produced through the use of charged particle accelerators, such as the cyclotron, or by neutron radiation in the chain-reacting pile, are characterized by β -ray activity. The unstable nucleus of these atoms in a long or short time will emit either a positively or negatively charged electron or β -ray and will be thus transformed into a stable isotope of another element. This β activity is sometimes also accompanied by γ -ray emission. Also β emitters are common among the naturally radioactive elements. Since apparatus designed for β -ray measurements is fairly sensitive to γ radiation, the same apparatus and often the same technique can be used for the measurement of both, especially where the data required are the relative amount of radioactive isotope present in unknown and standard.

Penetrating Power of Beta Rays

The penetrating power of the β radiation to be measured is an important factor influencing the design of equipment for β -ray measurements. If high sensitivity is desired, any window through which β particles are admitted into the measuring apparatus must be thin enough to permit a large portion to pass through it. Also the β -ray penetrating power will determine how

³⁹ W. C. Peacock and W. M. Good, *Rev. Sci. Instruments*, **17**, 255 (1946).

thick a sample can be measured before corrections for self-absorption will become important.

Discussions of the quantitative aspects of the absorption of β radiation are given by Rutherford, Chadwick, and Ellis,⁶⁰ Pollard and Davidson,⁶¹ Rasetti,⁶² and Feather.⁶³ A very useful summary of many of these data for β radiation as well as conversion electrons and x-ray and β -ray photons has been made by Glendenin.⁶⁴ Quantitative data on important β emitters are given by Libby.⁶⁵

The stopping power for β -rays of various materials, if measured in grams per square centimeter, is approximately independent for the lighter elements of the particular element or elements under consideration. More properly, it is closely proportional to the number of orbital electrons interposed, or Z/A , and this decreases somewhat per unit weight with heavier elements. For example, gold is about 20% less effective than aluminum as an absorber on a mass basis.⁶⁴

The maximum range of β -rays as a function of maximum energy is shown in figure 8a-b. The thickness of absorber that will decrease the number of emerging β -rays by $1/2$ is not a constant fraction of the total range but varies, depending upon the exact energy distribution of the β -rays, from about $1/5$ to $1/10$ of the absorber equivalent to the total range. For the purpose of deciding upon measurement techniques, $1/7$ is a useful approximate value.

Fortuitously, if the per cent of transmitted β -rays is plotted as the ordinate on semilog paper against absorber thickness linearly as the abscissa, the resulting plot will approximate a straight line. From this relationship, the data of table II have been derived, giving approximately the relationship between absorber thickness and per cent of β -rays transmitted. Table III gives the equivalent in milligrams per square centimeter of one-millimeter thicknesses of various substances.

The use of the following tables and graphs can be illustrated by an example: Suppose it is desired to find the thickness of mica that will transmit 90% of the β radiation incident on it from P^{32} . The peak β energy of P^{32} is 1.7 m.e.v. From figure 8a the maximum range of β -rays of this energy is 870 mg. per cm.² From table II the thickness of absorber that will transmit 90% of this radiation is 0.02×870 , or 17.4 mg. per cm.² Assuming an average value for white mica, the thickness of mica weighing 17.4 mg. per cm.² will be $17.4/235 = 0.061$ mm.

⁶⁰ E. Rutherford, J. Chadwick, and C. D. Ellis, *Radiations from Radioactive Substances*. Cambridge Univ. Press, London, 1930; Macmillan, New York, 1931.

⁶¹ E. Pollard and W. L. Davidson, *Applied Nuclear Physics*. Wiley, New York, 1942.

⁶² F. Rasetti, *Elements of Nuclear Physics*. Prentice-Hall, New York, 1936.

⁶³ N. Feather, *Proc. Cambridge Phil. Soc.*, **34**, 599 (1938).

⁶⁴ L. E. Glendenin, *Nucleonics*, **2**, No. 1, 12 (Jan., 1948).

⁶⁵ W. F. Libby, *Anal. Chem.*, **19**, 2 (1947).

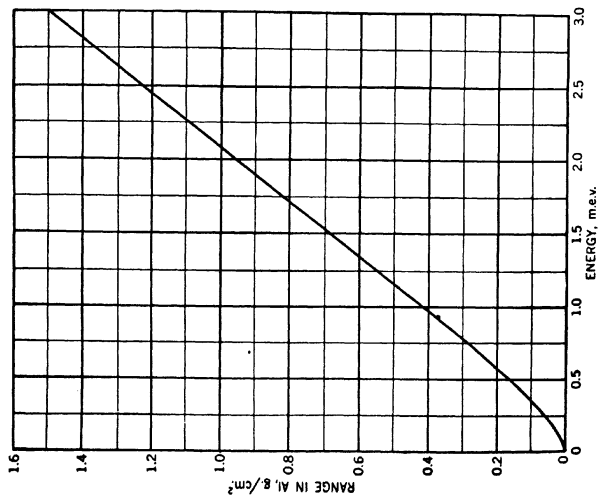


Fig. 8a.—Range of β -particles as a function of maximum energy.

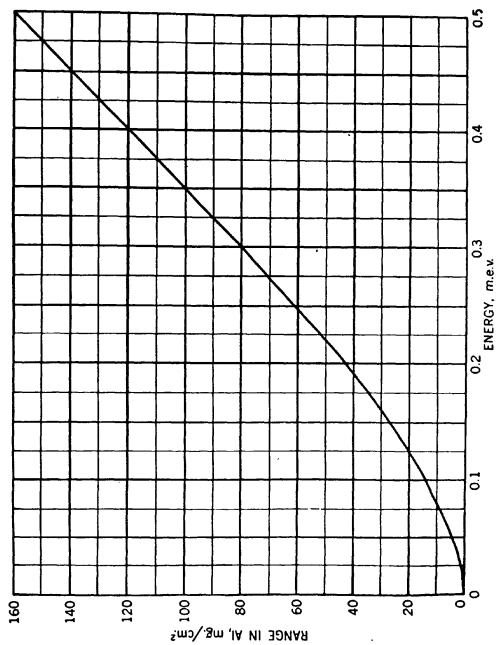


Fig. 8b.—Range of β -particles as a function of maximum energy.

As stated before, the values obtained by the use of these tables are only approximate. The actual absorption found is dependent upon the experimental geometry as well as the detecting device in use. If the sample measured is close to the window of the ionization-measuring instrument, β -rays passing through the absorber at angles considerably removed from the perpendicular to the absorber surface can be detected. Since, for such instances, the absorbing path will be longer, the proportion of incident β -rays transmitted will be less.

TABLE II
APPROXIMATE PER CENT OF β -RAYS TRANSMITTED BY AN ABSORBER^a

β -rays transmitted, %	Absorber thickness (maximum β range)	β -rays transmitted, %	Absorber thickness (maximum β range)
10.....	0.47	80.....	0.045
20.....	0.33	90.....	0.020
40.....	0.19	95.....	0.009
60.....	0.104		

^a Absorber thickness is given as a fraction of the absorber equivalent to the maximum range of the β radiation under consideration.

TABLE III
EQUIVALENT IN MILLIGRAMS PER SQUARE CENTIMETER OF ONE-MILLIMETER THICKNESSES (0.1 CC.) OF VARIOUS SUBSTANCES^a

Material	Weight of 0.1 cc., mg.	Material	Weight of 0.1 cc., mg.
Aluminum.....	270	Lead.....	1135
Beryllium.....	180	White mica.....	270-300
Magnesium.....	174	Pyrex glass.....	225
Iron.....	786	Water.....	100
Copper.....	894		

^a A 1-cm. layer of dry air at 0° C. and 76 cm. Hg pressure weighs 1.293 mg. per cm.².

With a large ionization chamber, the effective absorption will be measured as somewhat greater since the end of an ionizing path of a β -ray that would still register on a counter would produce only a few free ions in an ionization chamber.

V. MEASUREMENT OF HARD BETA RAYS

1. Apparatus

Because of the pronounced influence of the hardness of the β -ray on the design of apparatus for its measurement, it is convenient to divide appa-

ratus into two classes, for hard and soft β measurements. In the first class we shall place, for convenience, apparatus designed to measure with good efficiency β -rays with a maximum energy down to about 0.3 m.e.v. peak energy. This division is largely arbitrary. For example, the solution measuring technique to be described has been used to measure Ca^{48} in certain biochemical experiments. It was the method of choice because of the high accuracy easily obtained. Yet an increase in absolute sensitivity (not accuracy) of some fifty times could be obtained by a solid sample technique, a sensitivity that might be absolutely essential for the successful carrying out of other types of experiments.

Their wide commercial availability, high sensitivity, and the high accuracy obtainable under proper conditions make G-M counters the probable method of choice for hard β measurements. Two types of G-M tubes are generally available for this purpose. The first is a cylindrical, thin-walled tube, usually constructed of glass, the outer electrode consisting of a metallic film deposited on the interior of the glass, the second electrode consisting of a concentrically stretched tungsten wire (see Fig. 9).

The second or bell-type counter has the central electrode stiff enough (8 to 10 mil) so that it is supported at only one end. The window for entrance of β -rays, usually of mica, may vary in different designs from one to two inches in diameter. A design of a G-M tube of this type, due to Good, Kip, and Brown⁶⁶, is shown in figure 10. Other modifications are described in the literature and are available commercially.

The thin-walled type is less expensive and can probably be made with better electrical characteristics. The bell-shaped tube can be made the most sensitive. Including back-scatter, about 50% of the β -rays emitted from a thin sample can be detected with such a tube.

2. Preparation of Samples

There are three basic types of samples that may be prepared for activity measurements.

(1) The sample may be so thin that β -rays originating from the part of the sample farthest removed from the detecting device are not appreciably absorbed in passing through the remaining sample on the path to the G-M tube. In this case measurements will, in general, be proportional to total radioactivity and independent of the weight of the sample. The data presented in tables II and III and figure 8 can be used to calculate the approximate loss due to self-absorption in a sample of this type. To a first approximation an equivalent weight per square centimeter of other light elements will have the same absorbing power as aluminum. If the

⁶⁶ W. Good, A. Kip, and S. Brown, *Rev. Sci. Instruments*, **17**, 262 (1946).

sample is not uniformly distributed over the sample area, the effective self-absorption will not be the same as for uniform distribution.

(2) If the sample is thick compared with the penetrating power of the β -ray emitted so that none reach the counter tube from the side of the sample away from the counting tube, then the instrument will measure activity per unit weight independent of sample thickness.

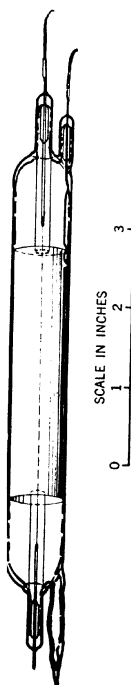


Fig. 9.—Tubular glass G-M counter.

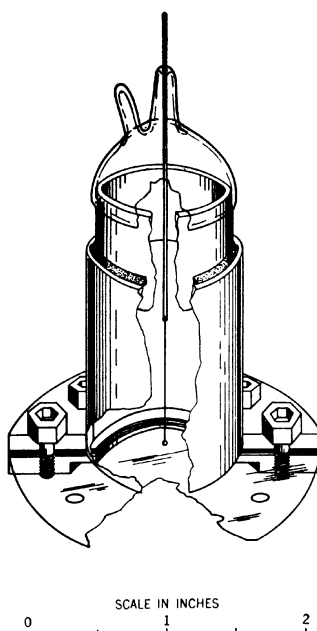


Fig. 10.—Bell-type counting tube.

(3) If the sample is of intermediate thickness according to the above criteria, for accurate measurements either this thickness must be reproducible or an experimentally made calibration curve must be available relating sensitivity to sample thickness.

In any case, samples and standard must in turn occupy identical posi-

tions during activity measurements and the radioactive material must cover a reproducible area, since, in general, the instrument sensitivity to any sample increment will depend upon its position. For the highest sensitivity the sample must be placed almost in contact with the counting tube. To reduce sensitivity in order to cover a wider range of activity the sample can be placed at a position farther away from the counter window. The accuracy of the desired result will, of course, determine the preciseness with which the above conditions must be fulfilled and often, therefore, the elaborateness of sample preparation.

Solid or precipitated samples may often be prepared by filtration, centrifuging, evaporation of solvent, or ashing in standardized containers. If the sample is collected on a filter paper, it is usually necessary either to clamp or cement a stiff backing to the paper during drying to prevent curling of the paper. Evaporation of solvents, particularly water, is likely to lead to an unhomogeneous deposit due to surface-tension effects. Addition of a small amount of detergent sometimes considerably improves the uniformity of deposit. Metals, such as iron,⁶⁷ may often be quantitatively electroplated on suitable planchets. Very good accuracy and reproducibility may often be obtained by spreading samples uniformly, as judged by eye, on suitable sample pans. A volatile liquid, such as ether or absolute alcohol, in which the material being measured is not more than slightly soluble, can often serve as an aid to transfer and even spreading. A more extensive description of techniques for solid sample preparation is given by Kamen.⁶⁸

In Chapter seven of *Isotopic Carbon, Techniques in the Measurement and Chemical Manipulation*^{68a} are described methods of preparing solid samples for radioactive carbon measurement that are also applicable to other elements.

One technique we have found useful is that of measuring samples in liquid form, usually as water solutions. A mica window, bell-type counter is satisfactory for this type of measurement. Standard sample containers are used with a circular cavity the diameter of the counting tube window, usually $1\frac{1}{4}$ to $2\frac{1}{4}$ inches and about $\frac{1}{8}$ inch deep. Stainless-steel cups are almost universally useful. For many solutions plastic containers, either machined or pressed, may be used. Two milliliters, or another appropriate amount, of unknown or standard is pipetted into the cup which is then brought to a reproducible position close to the tube window. For penetrating β emitters, such as P^{32} , absolute efficiencies as high as 10% in counting β disintegrations in the sample may be obtained.

⁶⁷ J. G. Gibson, J. C. Aub, R. D. Evans, W. C. Peacock, J. W. Irvine, Jr., and T. Sack, *J. Clin. Invest.*, **26**, 704 (1947).

⁶⁸ M. D. Kamen, *Radioactive Tracers in Biology*. Academic Press, New York, 1947.

Other methods of measuring radioactivity of liquid samples have been developed involving the use of dipping counters^{69, 70} or jacketed counters.⁷¹

3. Preparation of Standards

In most types of experiments with which the organic chemist may be concerned, it will be desirable to make the radioactive measurement in terms of a standard consisting of an aliquot of the originally prepared radioisotope. This standard should be prepared in a sufficient dilution so that its radioactivity is within the measuring range of the counter and can be easily counted with accuracy. If the time of measurement is prolonged over a period in which the change in counting rate due to radioactive decay is significant, a correction for this decay must be introduced in order to bring all measurements to an equivalent time. Even if the decay is known and can be allowed for by calculation, the standard provides a "known" on which it is advisable to have frequent measurements. If the decay curve is not known or the radioelement is being used in the form of a mixture of radioisotopes with differing half-lives, the decay of the standard as measured during the course of the experimental determinations gives an empirical curve from which decay corrections can be made. The decay of an individual radioactive isotope is such that a straight line is obtained if the activity is plotted on a logarithmic scale against time on a linear scale.

In order to check the sensitivity of the counting tube and the performance of the associated electrical circuits, it is desirable to have a permanent radioactive standard to measure on the counters. Potassium possesses a natural β activity with a peak β energy of 1.7 m.e.v. and a half-life of 4×10^8 years, adapting it well for use as a β -ray standard. For a solid standard, potassium nitrate may be made into a thick paste with water and dried in a sample pan to make a button a few millimeters thick. It is preferably kept afterward in a desiccator or warm oven. A nearly saturated solution of potassium acetate, made by dissolving three parts of acetate in two parts of water, is a convenient liquid standard.

A solid preparation of uranium salt is also satisfactory providing it is in equilibrium with its decay products UX_1 , UX_2 . Uranium itself emits only α -rays. Its apparent β activity is due to these degradation products

^{69a} Calvin, M., Heidelberger, C., Reid, J. C., Tolbert, B. M., and Yankwich, B. F., *Isotopic Carbon. Techniques in Its Measurement and Chemical Manipulation*. Wiley, New York, 1949; Chapman and Hall, London, 1949.

⁶⁹ W. F. Bale, F. L. Haven, and M. L. Le Fevre, *Rev. Sci. Instruments*, **10**, 193 (1939).

⁷⁰ A. K. Solomon and H. D. Estes, *Rev. Sci. Instruments*, **19**, 47 (1948).

⁷¹ A. R. Olson, W. F. Libby, F. A. Long, and R. S. Halford, *J. Am. Chem. Soc.*, **58**, 1313 (1936).

in equilibrium with it. One hard β -ray per second is the average emission of 1.5 micrograms of uranium. Uranium in solution must be used only with great caution because of the danger of UX_1 separation through adsorption or through chemical reactions not shared by uranium.

4. Statistical Fluctuations

Any individual radioactive atom is characterized by the fact that the moment of disintegration cannot be exactly predicted. However, from experiments with other atoms of the same isotope, an average life before disintegration can be established and therefore the odds of radioactive disintegration over any period of time calculated. This means that, over and above all other errors entering into an experiment, there will be an uncertainty due to the randomness inherent in a radioactive measurement. The researcher will, in general, wish to design his experiment so that errors due to statistical fluctuations discussed above will be kept below a predetermined level. Quantitative considerations of this subject appear in the literature.^{72, 73}

In connection with the use of the G-M counter the easiest estimate of this type that can be made is the standard error associated with the total number of counts recorded over a unit of time. If N is the total number of counts observed in a given period, the standard error, E_n , according to the binomial theorem will be:

$$E_n = \sqrt{N} \quad (1)$$

For example, assume that 200 individual counts have been recorded over a period of five minutes. Substituting 200 in the above formula, $E_n = \sqrt{200} = 14.1$. The standard error in per cent will be: $14.1/200 \times 100 = 7.06\%$. The following interpretation can be given to the above figure. There are approximately two chances in three, or a probability of 66%, that the activity of 200 counts per five minutes will be within 7% of the average value determined by counting over a period of time long enough to iron out statistical fluctuations of this type. Table IV gives the number of counts that must be recorded to keep the standard errors due to statistical fluctuations below certain percentage values of the whole count.

It is well known that, even in the absence of any normal sources of radiation, a G-M counting tube will not remain completely quiescent, but will continue to give a few discharges or counts per minute. This background or dark count is due to two sources: natural contaminating radioactivity of

⁷² E. Rodgers, *Phys. Rev.*, **57**, 735 (1940).

⁷³ L. J. Rainwater and C. S. Wu, *Nucleonics*, **1**, No. 2, 60 (Oct., 1947); **2**, No. 1, 42 (Jan., 1948).

TABLE IV
RELATION OF TOTAL NUMBER OF COUNTS TO STANDARD ERROR RESULTING FROM
RANDOMNESS OF COUNT DISTRIBUTION

Standard error, %	Number of counts	Standard error, %	Number of counts
0.5.....	40,000	5.....	400
1	10,000	10.....	100
2	2,500	20.....	25

the counter and surrounding material, and cosmic radiation. Although each can be substantially reduced by the wise selection of materials for counter construction and by massive shielding, it is not possible to eliminate this background entirely. Thus, in radioactive measurements one is primarily concerned not with the gross counting rate, but rather with the net increase in counting rate over the background count, often termed the net counting rate, and usually measured in counts per minute. The corresponding percentage probable error of the net count (here designated $E_n\%$) may be calculated as follows: If N = total counts taken of unknown, and T = time in minutes of counting unknown, the standard error in counts per minute of unknown = \sqrt{N}/T . Correspondingly, if n = total counts taken of background, and t = time in minutes of counting background, the standard error in counts per minute of background is \sqrt{n}/t . The standard error in the net count per unit time, K , will then be:

$$K = \left[\left(\frac{\sqrt{N}}{T} \right)^2 + \left(\frac{\sqrt{n}}{t} \right)^2 \right]^{1/2} \quad (2)$$

$$E_n\% = 100 \times \frac{K}{\text{net count/minute}} \quad (3)$$

For example, assume that a ten-minute count of an unknown gives 1000 counts, and that a twenty-minute background count gives 500 counts. Then the standard error in counts per minute of the net count is:

$$\left[\left(\frac{\sqrt{1000}}{10} \right)^2 + \left(\frac{\sqrt{500}}{20} \right)^2 \right]^{1/2} =$$

$$[(3.164)^2 + (1.12)^2]^{1/2} = 3.36$$

The net counting rate per minute is:

$$(1000/10) - (500/20) = 100 - 25 = 75 \text{ counts per minute}$$

with a standard error of 3.36 counts per minute:

$$E_n\% = 100 \times \frac{3.36}{75} = 4.5$$

The question often arises as to how long a sample should be counted and how long the background or dark count taken to keep errors from the random nature of this count below a certain predetermined percentage value. When the unknown radioactivity is several times the background in intensity, the data in table IV gave a sufficiently accurate answer. When the unknown is of the same order as the background in intensity, the background uncertainty effect is important and the question more complicated. The following considerations lead to a sufficiently accurate and easily applied solution. Using preliminary data, let M = counts per minute of unknown, T = time to be determined for counting the unknown, m = counts per minute of background, and t = time to be determined for counting background. Then E and e , the probable error in counts per minute of unknown and background, are, respectively:

$$E = \frac{(MT)^{1/2}}{T} = \frac{M^{1/2}}{T^{1/2}} \quad (4)$$

$$e = \frac{(mt)^{1/2}}{t} = \frac{m^{1/2}}{t^{1/2}} \quad (5)$$

At least for low counting rates of the order of background in single experimental determinations, it is good practice to determine unknown and background each to an accuracy that they will contribute equally to the accuracy of the final results. Therefore, we may set:

$$E = e = \frac{M^{1/2}}{T^{1/2}} = \frac{m^{1/2}}{t^{1/2}} \quad (6)$$

$$T/t = M/m$$

If K is the net standard error in counts per minute of unknown minus background, we can set $E_n\%$, the standard error in percentage of this counting rate, as:

$$E_n\% = \frac{K}{M - m} \times 100 \quad (7)$$

Since $N = MT$ and $n = mt$, equation (2) may be rewritten:

$$K = \left[\frac{M}{T} + \frac{m}{t} \right]^{1/2} \quad (8)$$

Substituting in equation (7) the above expression for K :

$$E_n\% = \frac{100}{M - m} \left[\frac{M}{T} + \frac{m}{t} \right]^{1/2} \quad (9)$$

Solving equation (6) for t and substituting this value in the above equation:

$$E_n\% = \frac{100}{M - m} \left[\frac{M}{T} + \frac{M}{T} \right]^{1/2} \quad (10)$$

Solving this equation for T :

$$T = \frac{20,000 M}{(E_n\%)^2 (M - m)^2}$$

An example of use of the above equation for the equation for weak samples is: A preliminary measurement gives a background counting rate of 50 counts per minute. The counting rate of the unknown is approximately 65 counts per minute. It is desired to know the net counting rate to a standard error of 5%. How long should unknown and background be counted?

$$T = \frac{20,000 \times 65}{(5)^2 \times (65 - 50)^2}$$

$T = 231$ minutes which is the time for counting the unknown. The time for counting the background is found by substituting in equation (6):

$$t = \frac{Tm}{M} = 231 \times \frac{50}{65} = 178 \text{ minutes}$$

The excessive time necessary for this measurement will be noted. Let us again assume that, by some method, perhaps heavy shielding of the counting tube with lead, the background counting rate, m , can be reduced from 50 to 20 counts per minute. The gross counting rate, M , would be similarly reduced to 35 counts per minute under these conditions:

$$T = \frac{20,000 \times 35}{(5)^2 \times (35 - 20)^2} = 124 \text{ minutes}$$

$$t = 124 \times \frac{35}{20} = 71 \text{ minutes}$$

The time necessary for this second determination, while still excessive, is about 55% of the first. It also illustrates the desirability, if low activities are to be measured, of a low natural background for the measurement apparatus, providing sensitivity to the desired radiations can be kept comparable.

The previous considerations have indicated the procedure with a maximum economy of effort as far as making a single determination is concerned. In many experiments, however, there will be several unknowns to compare with a standard. Here, since the same background count and standard activity will enter into each set of calculations, it is worth while to determine each of these to a higher degree of accuracy than the other unknowns. Also, the tendency to systematic errors from an inaccurate standard determination will be decreased.

It should be emphasized that all of the foregoing discussion has led only to methods of estimating and predetermining measurement error due only to the random nature of the radioactive disintegration process. No allowance has been made for errors due to any failure of the G-M tube to perform perfectly or to other types of error that may be introduced by, or be inherent in, any quantitative procedure. The above calculations only set an upper limit on the accuracy of the experimental results. Any actual experiment will necessarily be less accurate than calculated above. The same statistical considerations also apply to data obtained with proportional counters and linear amplifier particle counters, instruments in common use for α activity measurements.

Where total ionization-current integrators, such as electrometers or electroscopes, are used, it is not easy to predict the statistical uncertainty of data due to the inherent randomness of radioactive processes. Since the contamination with α emitters and cosmic-ray bursts can probably not be entirely avoided, and since an α -ray will produce an amount of ionization many times that of a hard β -ray, the background percentage uncertainty for such a chamber is likely to be considerably greater than for a G-M counter of comparative sensitivity.

5. Errors Associated with Counting Tube

Characteristic of all G-M counting tubes is the fact that the counting rate is a function of voltage. Figure 11 shows the counting rate of one mica window counting tube as a function of the voltage applied to it. The voltage range where counting rate is nearly independent of voltage is known as the *plateau* of the counter and may vary greatly depending upon the style and construction of the counter. A good tube should have a long plateau with little or no slope. On first using a counting tube, it is wise to determine the shape and extent of the plateau by counting background and a radioactive sample at various applied voltages and to operate it, thereafter, at a point at which the counting rate is as little as possible a function of voltage. It is not wise to raise the voltage for more than an instant to so high a point that the plateau of the counter characteristic

curve is passed and rapid spontaneous counting or gas discharge takes place in the tube, as some counters may be harmed temporarily or permanently by this treatment.

The plateau and counting threshold should be checked occasionally thereafter, since it may change with time because of the gradual exhaustion of the quenching gas, small leaks, internal surface changes, or other causes. Some counter plateaus show marked temperature dependence. Since with most counting tubes the counting rate is, at least to some extent, a

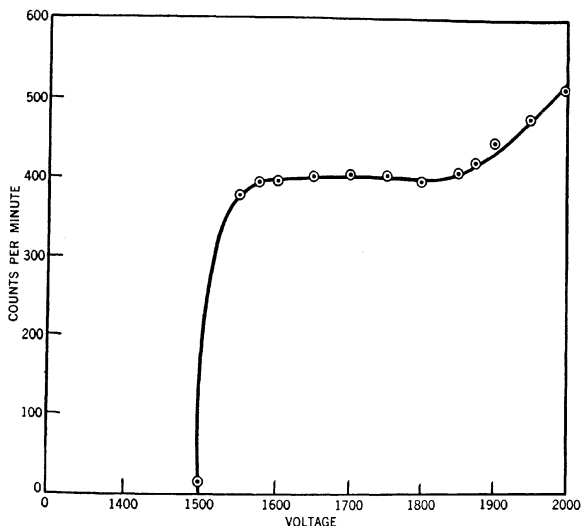


Fig. 11.—Counting characteristic curve of mica window counting tube filled to atmospheric pressure with argon plus alcohol; UX_2 beta source.

function of voltage, care must be taken that the applied voltage is a constant during the course of the experiment. Many voltage supplies have temperature coefficients such that the voltage may drift during a warming-up period. This change is not necessarily indicated by the voltmeter. Trouble due to this cause may sometimes be avoided by allowing a warming-up period for the apparatus of several hours or leaving it in continual operation.

The plateau of a counting tube is not necessarily the same at different counting rates or independent of the nature and direction of incidence of the ionizing particles being counted. It may also be dependent upon the

method used for quenching the counting discharge. It sometimes happens that, by counting at a rapid rate, a G-M tube is rendered unstable and if a background or low-activity sample is measured immediately afterward, the indicated activity will be too high due to spontaneous counts by the counting tube. Some G-M tubes are sensitive to visible or ultraviolet light, or become so with use, and respond to light by an increased background counting rate. Counters, therefore, are often provided with a light-tight box so that they operate in darkness. Unstable and therefore unsatisfactory counting tubes may give double, triple, or even larger bursts of counts. Sometimes this indicates only that the applied voltage is too high and satisfactory operation can be obtained at a voltage nearer the threshold.

It may also happen that an activity being measured is so high that in a significant number of instances a β -ray arrives before the previous count is complete and is then missed by the counter. Such failure may occur either in the counting tube or an associated quenching and recording electronic circuit.

It will be noted that the possibility of errors in measurement from some of the preceding effects will be kept at a minimum if: (a) in comparing an unknown and a standard the activity of the samples to be measured is kept closely similar or at least of the same order of magnitude; and (b) several measurements are made alternatively of standard and unknown. Frequent blank or background measurements will minimize effects due to contaminating radioactivity and apparatus instability.

6. Errors Associated with Electrical Circuit

As mentioned earlier, a common source of error in counting is that the activity will be so high that the instrument will miss recording certain counts due to inability of the electrical or mechanical system to respond individually to events too closely spaced in time. Such failure may have its origin either in the G-M tube and associated quenching circuit, in the amplifying and scaling circuit, or in the mechanical recorder.

Some electrical quenching circuits, particularly of the Neher-Pickering type, may be relatively slow in restoring full G-M tube voltage, so that even at rates of a few hundred counts per second, significant numbers of tube discharges may occur before full counting voltage has been regained. This means: (a) the tube may be part of the time operating at a condition of reduced sensitivity; and (b) some electrical pulses fed to the amplifier will be smaller than usual. Since the gain setting of the amplifier will determine whether such reduced pulses are counted or not, the ability of the G-M unit to respond at high counting rates will be dependent both upon the

G-M tube voltage setting which controls pulse height and upon amplifier gain setting. It is therefore advisable periodically to check the possibility of count loss at high counting rates and not to rely too much upon the counting characteristics of the unit tested under optimal conditions. One should have for use a standard of known activity as high as the highest unknown it is proposed to measure. Alternatively, a series of dilutions of a highly radioactive material can be prepared and, through measurements, a calibration curve made indicating deviations from linearity at high counting rates. This correction curve may be expected to change with time as a result of aging of radio tubes, possible changes in condenser and resistor values, as well as circuit adjustments such as those discussed above made by the operator; so it is important to keep activities low enough that this correction for missed counts does not exceed 10%. In preparing the calibration curve, allowances should be made, if necessary, for any self-absorption in the standards from which the correction curve is prepared.

Two occasional sources of fictitious counts may be mentioned. Inadequate high-voltage insulation may permit intermittent electrical discharges which may be heard as clicks, often periodic, in the speaker and which may register as counts on the recorder. Sometimes they appear only during periods of high humidity and may disappear if the electrical circuits are left in operation until the apparatus becomes warm. Electrical disturbance may be introduced from outside, over the a.-c. line, even though the counting and quenching circuit is electrically shielded, as is usually the case, and produces occasional counts or showers of counts on the recording apparatus. A shielded and grounded isolation transformer inserted in the a.-c. line will usually remove this source of trouble. The type of interference eliminators sold for use on radios seldom works here.

It is useful to have a switch located at the counting tube so that the high-voltage connection to the G-M tube can be easily opened to aid in determining whether such fictitious counts as those described above exist.

The voltage-regulating circuit on the high-voltage source should, of course, render it independent of any variations in line voltage encountered. The possibility remains, however, that other circuit components not similarly protected against line voltage variations may combine to make sensitivity dependent upon line voltage. A calibrated variable autotransformer is useful in checking the absence of such dependency.

VI. MEASUREMENT OF SOFT BETA RAYS

The comparatively recent widespread availability of C^{14} and S^{35} , and the still limited availability of H^3 , mean that the development of precise means of abundance determinations for these isotopes is still in very active

progress. The basic principles, of course, are the same as for isotopes emitting more penetrating β radiation, but the actual method of choice will depend not only on the element under consideration but also on the nature of the investigation undertaken. Therefore, the isotopes mentioned above will be considered individually rather than as a class.

1. Carbon¹⁴ Determinations

The peak energy of the β radiation from C¹⁴ is 0.145 m.e.v., the average energy about 0.058 m.e.v. Table V shows the per cent of perpendicularly incident C¹⁴ β -rays that will penetrate various amounts of mica and, to a good approximation, other elements.

A. SOLID SAMPLE PREPARATION AND MEASUREMENT

As discussed previously, two conditions for activity measurement on solid samples exist when the reproducibility of measurement is not greatly in-

TABLE V
TRANSMISSION OF C¹⁴ β RADIATION THROUGH MICA^a

Mica thickness, mg./cm. ²	Transmission, %	Mica thickness, mg./cm. ²	Transmission, %
1.5.....	80	7.6.....	20
2.4.....	60	10.8.....	10
4.2.....	40		

^a Mica of 0.0001 inch thickness weighs about 0.675 mg. per cm.².

fluenced by even minor nonuniformities in the distribution of the sample: (1) The sample is so thin that all β -rays emerge in the direction of the counter window with negligible absorption. (2) The sample is thick enough that few or no β -rays from the back of the sample are able to emerge and penetrate the counter window. In this case the activity will be proportional to the concentration of C¹⁴ per gram of sample and will be little influenced by the total sample weight.

The first method of sample preparation has the practical disadvantage in many types of research, that to fulfill this condition only a fraction of a milligram of carbon can be measured. Ordinarily this will represent very inefficient use of samples that will usually contain several milligrams or more of carbon.

This low penetrating power of C¹⁴ β -rays also indicates that for most work it will be necessary to prepare the carbon as an isolated compound of known composition for measurement purposes; the most common preparation is as barium carbonate. For practical purposes an "infinite

thickness" sample of barium carbonate weighs about 10 mg. per cm.² of which 0.6 mg. represents carbon content. If a sample of this type is placed close to a mica window of 1.5 mg. per cm.², about 5% of the total β particles emitted in the sample will penetrate the window.

In the selection of a counting tube, assuming the possible counting tubes all have desirable electrical characteristics, the specifications of primary importance are window thickness and diameter. The area of a sample that can be effectively used, and therefore the carbon content of the sample that can be measured, is proportional to the window area. Thus a counting tube with a window diameter of 1.75 inches and thickness corresponding to a weight of 1.5 mg. per cm.², will record about 5% of the disintegrations that occur in a 150-mg. barium carbonate sample containing 9 mg. of carbon.

By the use of counters in which the solid, dry sample is placed inside the counting tube, the window absorption effect can be largely eliminated. One such tube known as the screen-walled counter has been designed by Libby and Lee.⁷⁴ Also, β counters have been constructed similar to the proportional α counters developed by Simpson⁴³ in which the counter operates at atmospheric pressure, the counting gas is purified by continual renewal, and the sample to be counted is introduced through an air lock. The commercially available Nucleometer is an instrument of this latter type. Occasionally the higher sensitivity thus obtainable, perhaps two or three times that of the thin mica window counter, will compensate for the more difficult and time-consuming manipulation necessary with some of these counting tubes.

Ionization chambers can in theory perform as well as or better than counters in the measurement of solid samples. This is due to the higher specific ionization of the carbon β -ray as compared with the ionizing particles of cosmic radiation and the β -rays and recoil electrons produced by unavoidable radioactive contamination. Thus, referred to the reading for a C¹⁴ sample, the background will be lower in terms of integrated ionization current than in counts of ionizing particles. In practice, however, users of ionization chambers have measured C¹⁴ as gaseous CO₂, since in this case the large self-absorption effects can also be eliminated.

For the preparation of barium carbonate, organic samples can be oxidized by a standard combustion train for carbon determination or by the wet oxidation method of Van Slyke and Folch.⁷⁵ After dispersion through a fritted glass disk, the CO₂ is absorbed in sodium hydroxide solution. Barium carbonate is precipitated from this solution by barium chloride. The barium carbonate is uniformly dispersed on a counting sample holder

⁷⁴ W. F. Libby and D. D. Lee, *Phys. Rev.*, **55**, 245 (1939).

⁷⁵ D. D. Van Slyke and J. Folch, *J. Biol. Chem.*, **136**, 509 (1940).

by one of the sample preparation methods described earlier, dried to constant weight, and is ready for radioactivity measurement. Weight determinations on the precipitated barium carbonate also can give a quantitative carbon analysis for the sample. If the carbon content of the organic material is less than that desirable for radioactive determination, additions as sodium carbonate are conveniently made at an appropriate point. Precautions to exclude atmospheric CO_2 are necessary if high accuracy is desired.

More detailed discussions of the above method as adapted to their use by experienced investigators are available in the literature.^{68, 68a, 76-79} It has been shown that to prevent loss of C^{14} from stored, apparently dry barium carbonate, atmospheric water vapor, CO_2 , or both should be excluded.⁸⁰

B. GASEOUS SAMPLE PREPARATION AND MEASUREMENT

Design and Use of Ionization Chambers.—If the radioactivity of C^{14} is measured as a gas, usually CO_2 , it is possible to escape to a great extent from two important limitations of the solid sample method. These limitations for measuring solid samples are the comparatively low counting efficiency, below 5%, usually obtained, and the small amount of carbon that can be effectively used for measurement, around 5 to 10 mg. By contrast a one-liter ionization chamber at atmospheric pressure will hold 540 mg. of carbon as CO_2 . Also the average β -ray of C^{14} will have a path length from the site of origin of about 4 cm. so that in an ionization chamber of this size close to the maximum ionization will be produced by C^{14} β -rays before they strike the chamber wall.

Also, as mentioned earlier, in a current-integrating instrument the C^{14} β -ray will produce a many times larger ionization-current increment than most individual cosmic-ray particles or β - and γ -rays of outside origin. By contrast, in a G-M counter each gives an equivalent count. Of course, α -rays will in turn produce ionization-current increments around twenty-five times the contribution of a carbon β -ray. But α contamination can be reduced to very low values. Also dynamic condenser electrometers can be made with sufficient sensitivity that α -particle contributions can be

⁷⁶ D. B. Melville, J. R. Rachele, and E. B. Keller, *J. Biol. Chem.*, **169**, 419 (1947).

⁷⁷ W. G. Dauben, J. C. Reid, and P. E. Yankwich, *Anal. Chem.*, **19**, 828 (1947).

⁷⁸ H. E. Skipper, C. Bryan, L. White, and O. F. Hutchison, *J. Biol. Chem.*, **173**, 371 (1948).

⁷⁹ D. W. Wilson, A. O. C. Nier, and S. P. Riemann, eds., *Preparation and Properties of Isotopic Tracers*. Edwards, Ann Arbor, 1946.

⁸⁰ W. D. Armstrong and J. Schubert, *Science*, **106**, 403 (1947).

noted and individually subtracted from an integrated ionization-current measurement.

Even the relatively insensitive Lauritsen electroscope can be used for ionization chamber measurements of C^{14} providing the chamber is designed for a minimum electrical capacity of the collecting electrode. Henriques and Margnetti⁸¹ have constructed such a chamber from a 200-ml. quartz flask silvered over a portion of its internal surface. They state that such an apparatus, if shielded with two inches of lead, would detect 40 disintegrations per minute of C^{14} present as CO_2 . This unit is normally used at a CO_2 pressure of two atmospheres and under these conditions accepts 0.24 gr. of carbon as CO_2 .

Use with the above apparatus of the improved Lindemann electrometer now available should increase the speed of low activity and background measurements which may be inconveniently long with the Lauritsen electroscope.

For routine work in which many determinations are to be made, use of one of the dynamic condenser electrometers has the advantage that written activity records are made with little attention from the operator. Also, the voltage sensitivity of these instruments is such that it is not necessary to keep the electrical capacity of the ion-collecting system at the irreducible minimum. This makes possible the design of better ionization chambers from the electrical standpoint. "Soak-up" effects and other causes of potential erratic operation can be eliminated. Figure 12 shows the design of a 1300-ml. ionization chamber for use with the ballistic vane electrometer, a version of the dynamic condenser electrometer previously mentioned. Its design illustrates principles to be kept in mind in the design of any other ionization chamber. Guard ring construction is used, that is, a grounded metal segment completely isolates the collection voltage. Grounded shields are interposed so that the collecting electrode insulator does not "see" anything at the ion-collecting voltage potential. Also, shields prevent the collecting electrode "seeing" any collecting voltage insulators. The "dead space" is small so that little chamber volume is wasted insofar as furnishing collectable ions is concerned. No grounded surface is exposed to the main ion-collecting volume to act as a parasitic collector of ionization current. The active chamber wall is of virgin copper sheet, a material with low natural α -active contaminants. It is readily demountable so that it can be well cleaned with emery cloth, a procedure useful in reducing natural surface contamination. It is mechanically isolated from the outer pressure-bearing wall so that its position is unaffected by pressure changes during evacuation and refilling of the chamber. The

⁸¹ E. C. Henriques, Jr., and C. Margnetti, *Ind. Eng. Chem., Anal. Ed.*, **18**, 417 (1946).

chamber is electrically well shielded. The central insulator, in this case lucite, was carefully machined with coolant to avoid surface strains, then highly polished to reduce surface leakage. Before assembly all parts were cleaned with ether, the collecting electrode lead-through insulation with particular care, and the insulating surface not touched thereafter during assembly. If neoprene or other rubber is used for gasket purposes, as it is in

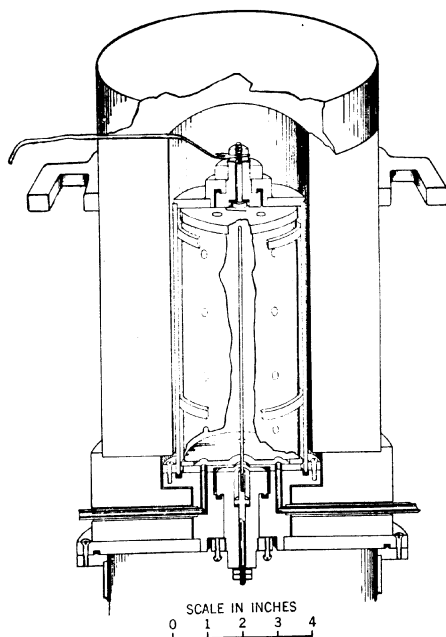


Fig. 12.—Ionization chamber.

this chamber, exposure to CO_2 should be as small as possible to reduce CO_2 absorption, and the chamber should be filled with nonradioactive CO_2 when not in use for actual measurements. The whole chamber, and especially the ion-collector electrode insulator, is of fairly massive construction so that during evacuation mechanical deformation will not give rise to electrical charges producing fictitious activity readings. In actual operation, when shielded with two inches of virgin lead and used with the ballistic vane electrometer, the background ionization current is equivalent to not more than 200 C^{14} disintegrations per minute. A net activity of 400 C^{14}

disintegrations per minute contained in 0.55 g. of carbon can be determined to an accuracy of 10% in a period of 20 minutes.

In contrast, this activity contained in a solid sample of 9 mg. of carbon as barium carbonate would give a counting rate with a G-M counting tube of not more than 20 counts per minute above background, determinable to the same accuracy in about the same period. Thus, where large samples (the order of 0.5 g. carbon content) are available, the gas chamber has a useful sensitivity about 70 times that of the thin window G-M counter.

An ionization chamber suitable for routine use with the Lindemann electrometer has been described by Janney and Moyer.^{81a} Jesse and coworkers^{81b} report on the performance of an ionization chamber adapted for use with the vibrating reed electrometer. The ionization chamber described above can also be adapted for use with these instruments. Batteries should be used as the source of chamber potential source since even the best regulated a.-c. supplies have fluctuations producing unnecessary uncertainty in low activity readings. As in the preparation of solid samples of barium carbonate from organic material, either dry combustion⁸¹ or the wet oxidation method of Van Slyke^{75, 78} can be used to generate CO₂.

The wet oxidation method has proved very convenient in handling dry organic samples of the order of 1 g. dry weight; 50 ml. of the oxidizing solution are necessary per gram of sample. Moist samples may also be oxidized providing 50 ml. of the complete oxidizing solution per ml. of water are used in addition, together with proportional additions of KIO₃.

The CO₂ produced is carried by tank nitrogen through a short glass bead column filled with concentrated H₂SO₄ and further dried either with phosphorus pentoxide or a glass trap cooled with dry ice in acetone. It then passes through a metal trap cooled with liquid nitrogen into which the CO₂ is condensed. Construction of this trap is shown in figure 13. It provides a long path so that CO₂ is effectively

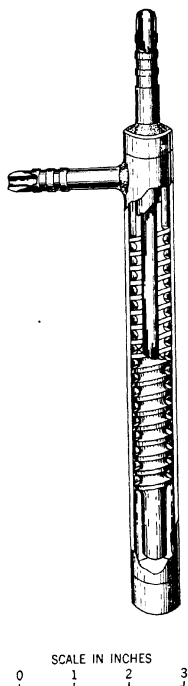


Fig. 13.—Trap for CO₂ condensation.

^{81a} C. D. Janney and B. J. Moyer, *Rev. Sci. Instruments*, **19**, 667 (1948).

^{81b} W. P. Jesse, L. O. Hannum, H. Forstat, and A. L. Hart, *Phys. Rev.*, **71**, 478 (1947).

removed even at high rates of CO_2 generation, yet it is resistant to plugging even at slow rates of condensation.

Following the complete washing of the CO_2 into the liquid-nitrogen-cooled trap, this trap is evacuated and connected to the previously evacuated ionization chamber, through an electrostatic ion trap, following the design of Evans⁸² or Henriques and Margnetti.⁸¹ The use of such an ion trap is essential. On removing the liquid nitrogen the CO_2 changes to the gas phase, distributing itself between trap and ionization chamber according to their relative volumes. Inert tank CO_2 is then bled into the chamber through the ion trap bringing the pressure up to atmospheric.

It is important to exclude air from the apparatus and to use nitrogen which has been previously stored for several weeks for CO_2 transport instead, since it is found that the normal radon content of air is such that if air is used, enough radon is condensed into the liquid-nitrogen-cooled trap, and thus introduced into the chamber, to produce ionization equivalent to 200 to 300 C^{14} disintegrations per minute. Also, commercial barium salts are found to contain enough radium that the radon therefrom is easily detectable. Thus barium carbonate must be used with caution as a source of CO_2 gas for measurement purposes at low levels of C^{14} concentration.

In practice it is found that approximately one sample per hour can be oxidized and measured with this apparatus; the following sample is oxidized and condensed in the nitrogen-cooled trap while data on the preceding sample are automatically recorded.

G-M Counters for Gaseous Samples.—That with suitable precautions gaseous CO_2 can be introduced directly into a G-M counting tube and individual C^{14} disintegrations counted, has been shown by Miller.⁸³ Counting efficiency is close to 100%. Carbon disulfide vapor to a pressure of 2 cm. of mercury is added to the tube to improve the counting qualities of the gas, and a modified Neher-Pickering quenching circuit used. A regulated voltage source up to 5000 volts is necessary if CO_2 pressures up to 50 cm. of mercury are to be used. In a tube that will hold 0.18 g. of carbon as CO_2 , Miller reports a background count of 300 counts per minute.

An adaptation of essentially the above technique to routine measurement of 50-mg. organic samples has been reported in detail by Skipper and co-workers.⁷⁸ Their tube has a volume equivalent to 0.11 g. of CO_2 at 35 cm. pressure. The background with 2 inches of lead shielding is 45 counts per minute.

Since most solid sample methods of measuring C^{14} also involve the oxidation of organic material and quantitative collection of CO_2 gas, it seems that methods in which CO_2 is measured in the gaseous form have the fun-

⁸² R. D. Evans, *Ind. Eng. Chem., Anal. Ed.*, **4**, 233 (1933).

⁸³ W. W. Miller, *Science*, **105**, 123 (1947).

damental merit of eliminating the necessity of an important portion of the work necessary in solid sample techniques.

C. CONSIDERATIONS GOVERNING CHOICE OF METHOD

At the moment, apparatus is commercially available only for solid sample measuring techniques. Bell-type, mica window G-M tubes have the greatest versatility of any radioactivity measurement apparatus. G-M counters still occasionally need competent electronic servicing. If such assistance is unavailable, use of a Lauritsen electroscope as described for radioactive sulfur⁸⁴ may be the method of choice.

For an intensive program of work where over-all cost of many reliable measurements, rather than the initial cost of apparatus, is the primary consideration, gas measurement methods should be considered. Use of the Lauritsen electroscope as described by Henriques and Margnetti⁸¹ is the least expensive. Use of an ionization chamber with a dynamic condenser electrometer can perhaps best be adapted to accurate speedy routine use. It is probably initially also the most expensive method. The CO₂ filled counter technique as developed by Miller⁸³ is perhaps the most sensitive for samples of 50 mg. of carbon or less, and intermediate in cost. When samples of ample radioactivity are available, it is probably more difficult to obtain highly accurate results with the gas-filled counter than with the ionization chamber.

2. Measurement of Sulfur⁸⁵

The peak β energy of S³⁵ is so close to that of C¹⁴, 0.17 as compared with 0.145 m.e.v., that the physical problems of measurement are essentially identical. The methods of measurement so far developed have involved solid samples. Two reasons account for this: (1) In biological work the sulfur concentrations encountered are much lower than for carbon so that efficient methods of measuring samples of more than a few milligrams are not as useful. (2) Sulfur is probably not as readily converted to an easily handled gas for ionization chamber use as is carbon; although SO₂ is more reactive than CO₂, the possibility of designing satisfactory apparatus exists for its use in gas phase measurements.

For solid sample measurement sulfur may be precipitated as benzidine sulfate⁸⁴ or as barium sulfate, after previous oxidation to sulfate by the Carius method⁸⁵ or by wet digestion with nitric acid followed by perchloric

⁸⁴ F. C. Henriques, Jr., G. B. Kistiakowsky, C. Margnetti, and W. Schneider, *Ind. Eng. Chem., Anal. Ed.*, **18**, 349 (1946).

⁸⁵ J. B. Niederl, H. Baum, J. S. McCoy, and J. A. Kuck, *Ind. Eng. Chem., Anal. Ed.*, **12**, 428 (1940).

acid. The physical methods of solid sample preparation described for barium carbonate can, in general, be used for sulfur precipitated as benzidine or barium sulfate.⁶⁷

If high sensitivity is essential in handling sulfur samples larger than 10 mg., reduction to elemental sulfur and measurement in this form should perhaps be considered.⁸⁶

3. Measurement of Radioactive Hydrogen

Two characteristics of H^3 or tritium are of importance in setting up measurement techniques. The maximum β energy is so low, about 0.015 m.e.v., that even the most penetrating β will travel only through a few millimeters of gas and an equivalent amount of other substances. Thus, even the thinnest counter window is essentially opaque to these radiations, in fact, self absorption corrections are so great that any solid sample technique has little to recommend it; H^3 will preferably be measured as a gas.

Also, H^3 is unique among radioactive isotopes in that it is much heavier, about 300%, than the normal, nonradioactive isotope. This great mass difference means that in sample preparation for radioactivity measurement, precautions must be taken that the hydrogen isotopes are not preferentially separated during sample preparation. One method of accomplishing this is to design the preparatory procedure so that all of a sample is converted into the gas introduced into the counter or ionization chamber. Thus, electrolysis of water as a means of producing hydrogen is difficult to use in a satisfactory manner because of the serious fractionation that occurs during electrolysis and the difficulty in carrying this reaction to completion. More generally, this means that H^3 can be used as a quantitative hydrogen label or tracer only with very great caution. In fact, its greatest potential use is not as a hydrogen but rather as a carbon tag. Linked stably to carbon, the mass difference from ordinary hydrogen becomes relatively unimportant. The synthesis of desired compounds may in many instances be much easier with hydrogen than with carbon labeling.

Henriques and Margnetti⁸⁷ have described a general procedure for the preparation of gaseous hydrogen from organic material for tritium activity determinations. Hydrogen present in the sample is converted to water by oxidation combustion, then collected and weighed in the frozen state. After dilution, if necessary with nonradioactive water, it is reacted with magnesium amalgam to form hydrogen. This hydrogen is introduced into the ionization chamber. Using an ionization chamber attached to a

⁸⁶ H. Borsook, E. R. Buchman, J. B. Hatcher, D. N. Yost, and E. McMillan, *Proc. Natl. Acad. Sci. U. S.*, **26**, 412 (1940).

⁸⁷ F. C. Henriques, Jr., and C. Margnetti, *Ind. Eng. Chem., Anal. Ed.*, **18**, 420 (1946).

Lauritsen electroscope in a manner similar to their method for C^{14} determinations, measurement of hydrogen with an activity of 660 β particles per minute to an accuracy of 1.4% average deviation is reported. Their chamber accommodates hydrogen equivalent to that in 240 mg. of water.

Alternative methods for activity determinations in a manner similar to C^{14} might involve the use of the Lindemann or other quadrant electrometers or a dynamic condenser electrometer.

Also, hydrogen can be introduced directly into a G-M counting tube for measurement. The addition of about 1 cm. of mercury pressure of ethyl alcohol plus the use of a good electronic quenching circuit provides quite satisfactory counter operation. This is probably the most sensitive method for tritium determinations. The use of a counter-tube filling of helium, alcohol, and hydrogen has been reported.⁸⁸ For an additional discussion of H^3 measurement techniques, see Kamen.⁶⁸

4. Measurement of Other Soft Beta Emitters

The measurement of 90 day half-life arsenic in solid samples has been described by Henriques and Margnetti,⁸⁹ of Ca^{43} by Kamen.⁶⁸ Principles involved are similar to those of C^{14} and S^{35} measurements.

5. Measurement of Conversion Electrons

Conversion electrons are distinguished from other β -rays only by the fact that those originating from one atomic species all have the same energy rather than the energy distribution characteristics of β -rays of nuclear origin. The penetrating power of conversion electrons as a function of energy is given by Rutherford, Chadwick, and Ellis.⁹⁰ Since conversion electrons are initially homogeneous in energy, reduction in intensity by an absorber tends to be a linear function of absorber thickness. This contrasts with β -rays where the log of the β -ray intensity transmitted is inversely proportional to the absorber thickness. It follows that methods of detecting and measuring conversion electrons are essentially the same as those used for β -rays of similar penetrating power. The characteristic x-rays that almost invariably accompany electron formation occasionally also provide useful means of activity measurements.

VII. MEASUREMENT OF GAMMA- AND X-RAY ACTIVITY

In their interaction with matter, γ -rays lose energy predominantly by three processes that result in the production of fast-moving electrons similar

⁸⁸ S. Gurin and A. M. Delluva, *J. Biol. Chem.*, **170**, 545 (1947).

⁸⁹ F. C. Henriques, Jr., and C. Margnetti, *Ind. Eng. Chem., Anal. Ed.*, **18**, 415 (1946).

to the β radiation of nuclear origin: (1) the photoelectric reaction; (2) the production of Compton recoil electrons; and (3) pair production of positive and negative electrons. Of these three processes, the production of recoil electrons is likely to predominate in the radiation from radioactive elements.

In tracer experiments, the same apparatus and essentially the same technique may often be used for both β - and γ -ray measurements. Often, in fact, for elements producing both β and γ radiation, such as Na^{24} and Fe^{59} , the ionization measured is contributed in part by β particles and by γ quanta. If it is desired to measure γ radiation exclusively, a shield may be inserted between the source and radiation detector thick enough to prevent the passage of the most penetrating β particles.

Due to the penetrating nature of most γ radiation, most quanta will pass completely through the ordinary counter or ionization chamber designed for β -rays without producing ionization. As a result of this effect, the efficiency of detecting γ quanta will be small, at most a few per cent of the β -ray measuring efficiency easily obtained. By using large G-M counters or ionization chambers, the sensitivity to γ radiation may be increased. This may also be accomplished by the use of heavy vapors or gases under pressure in an ionization chamber. However, once the chamber is large enough to read the background of ionization due to cosmic radiation quickly and accurately, little further is gained by increased size since the blank or background measurement will increase proportionally with the unknown, and no gain in usable sensitivity results.

Evans and Mugele⁹⁰ have described a G-M counting tube that, by means of a rough inner wall obtained by using copper screen, gives an increased sensitivity of two or three for γ -rays over the more penetrating cosmic radiation. Further increase in sensitivity may be obtained by plating this screen with bismuth.⁶⁶

One advantage of the use of γ -rays in tracer studies is that corrections for absorption, in comparing unknown and standard, often become small or negligible. This made possible, for example, the work of Hertz⁹¹ in measuring the amount of radioiodine deposited in the thyroid of unoperated human patients.

Measurement of Characteristic X-Radiation

In certain radioactive isotopes, for example, the four year half-life isotope of iron, Fe^{55} , radioactive decay involves K-electron absorption by the nucleus, rather than the emission of β -rays. The K-electron capture results

⁹⁰ R. D. Evans and R. A. Mugele, *Rev. Sci. Instruments*, **7**, 441 (1936).

⁹¹ S. Hertz, *J. Applied Phys.*, **12**, 313 (1941).

in characteristic K x-ray production of very low penetrating power as well as emission of low-energy Auger electrons. In general, the same techniques are useful in detecting such radiation as is useful for soft β -rays. There is one important deviation. The mass absorption coefficient for soft x-rays is proportional to about the fourth power of the atomic number of the absorbing material, rather than almost independent of this factor as is the case for β radiation. Thus, if maximum penetration is desired, a window of as low atomic number as possible is used; in practice this is usually beryllium. Special G-M tubes are available with 70 to 80% quantum efficiency for x-rays in the region of 1.5 Å.

VIII. MEASUREMENT OF ALPHA-RAY ACTIVITY

The outstanding characteristic of the α -ray, as concerns methods of measurement, is its low penetrating power. Figure 14 gives the range of

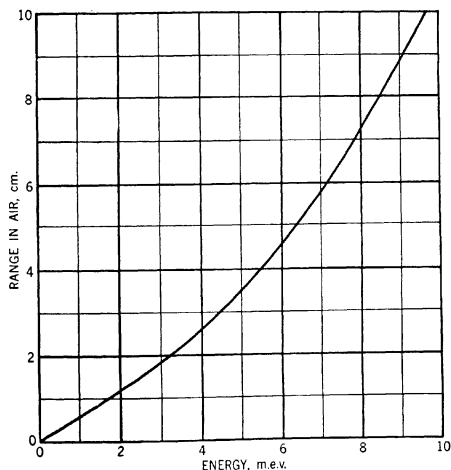


Fig. 14.—Alpha energy range curve.

α -rays in air as a function of energy. The stopping power of other materials can be derived from Bragg's rule which states that the mass stopping power is inversely proportional to the square root of the atomic weight. Unlike β -rays, α -rays from one isotope have predominantly the same energy and range. Also, the energy of α -rays from all substances fall predominantly in the range from 3 to 8 m.e.v. For quantitative data on α radiation

and the naturally occurring α emitters, see especially Rutherford, Chadwick, and Ellis⁶⁰ and Rasetti.⁶²

1. Preparation of Samples for Measurement

The general nature of the problems encountered in preparing α -active samples for measurement is the same as for soft β emitters such as C^{14} . If the mass of the sample to be measured in solid form is small, it can sometimes be distributed in such a thin layer that at any point the self-absorption of the sample will be negligible. The thickness is of the order of 0.1 to 0.3 mg. per cm.² evenly distributed for oxides of the heavy metals. With large samples of uniform or known composition, the preparation to be measured may be made so thick that radiation from the back of the samples does not reach the surface. With two samples of equal surface areas, even with different sample weights, the true activity per unit weight of material under these conditions can be kept proportional to the measured activity. It is essential that the chemical composition of the unknown and standard be equivalent in α -ray absorbing properties. This can usually be satisfied at a sacrifice in sensitivity even though the unknown and standard are of different or unknown composition, providing the α -active element is the same, by dispersing them through an amount of the same inert material large in comparison with the substances measured.

2. Apparatus

In principle, any of the types of apparatus previously described may be used for α -ray measurements provided the air path between source and ionization chamber is short enough and the entrance window thin enough to permit entrance of the rays. A G-M counter with a 1.5 mg. per cm.² mica window will count about 20% of the α -rays from a thin α -active sample placed almost in contact with the window.

The linear amplifier, proportional counter, and ionization chamber all have sensitivity advantages over the G-M counter in the following respects. There is in each an unavoidable background of ionizing particles due to cosmic-ray effects and radioactive contamination of apparatus. In the G-M counter each ionizing particle will register as a count even though, as is generally true, most other ionizing particles produce many times fewer ions than does the α -ray. On the other hand, the linear amplifier and proportional counter can be operated so as to discriminate between α particles and other ion producers, such as β -rays, and record only the former. Also, the ionization chamber, while not able to discriminate entirely between sources of ionization, weights its measurements of radiation intensity according to the ionizing power of the radiation and thus is, from this point of view, preferable to the G-M counter.

The Simpson proportional counter⁴³ and the parallel plate linear amplifier such as the one described by Young⁴² are probably the most widely used instruments for α -activity determinations on solid samples. Each of these instruments will count, from a sample thin from the standpoint of self-absorption, to an accuracy of a fraction of 1%, exactly 50% of the α -rays emitted by a flat sample. Each can count α -rays in the presence of considerable β activity. The proportional counter is superior in the amount of intermixed β activity that it can tolerate, the order of 5×10^3 disintegrations per minute without interference with α counting, and the high rate of α counting that can be achieved, 10^5 counts per minute, before losses exceed 1%. Linear amplifier α counters have been designed to receive samples of much larger area than proportional counters so far constructed for precision measurement purposes. Instruments of both types are commercially available.

If powdered samples are measured, it is desirable to provide a binder to prevent apparatus contamination. Spraying with collodion in ether so limited that equivalent absorption does not exceed 0.1 mg. per cm.² has been found satisfactory.⁴³

Rough samples, minute hairs, excess moisture, etc., can give spurious counts. Absence of such effects should be checked by preparation of a suitable number of nonradioactive but otherwise entirely equivalent blanks. If "thick" rather than "thin" samples are measured, the α pulses from the amplifier will be of all possible sizes below the maximum. Under these conditions linear amplifiers and proportional counters will have no true plateau, and the equivalence of the number of counts recorded will depend upon absolute constancy of the amplifying and discriminating circuit and should be checked by frequent measurement of thick α standards.

Gas samples containing α activity can be measured either with a linear amplifier or by one of the ionization-chamber methods. For low activities the vibrating reed electrometer set up to detect single α particles appears a promising method.

Alpha-activity measurements of gaseous samples have been used primarily for radon measurements either to evaluate industrial health hazards or as a method for quantitative radium analysis. The use of ionization chambers and electrometers for this purpose has been described by Evans and coworkers,⁹³ and Hess and McNiff.⁹⁴ Curtiss and Davis report the use of a gas α counter.⁹⁵

⁹² H. D. Young, *Manhattan District Declassification Code 233* (Aug. 27, 1946)

⁹³ R. D. Evans, *J. Ind. Hyg. Toxicol.*, **25**, 253 (1943).

⁹⁴ V. F. Hess and W. T. McNiff, *Am. J. Roentgenol. Radium Therapy*, **57**, 91 (1947).

⁹⁵ L. F. Curtiss and F. J. Davis, *J. Research Natl. Bur. Standards*, **31**, 181 (1943).

IX. DETERMINATION OF ABSOLUTE BETA ACTIVITY

It is often necessary or desirable to determine the actual β activity of a sample in terms of disintegrations per minute in which β -rays are produced. Such measurements, if done other than by comparison with a known sample of the same isotope, are almost invariably made using a particle-counting device, usually a G-M counter. The use of an ionization chamber will usually involve a difficult correction for the different ionizing power of the ionizing particles of the unknown as compared with the standard radioactive source.

Beta-ray standards of known activity are now available from the Bureau of Standards for this purpose. Also uranium oxide or a uranium salt deposited in a uniform thin layer can be used as such a standard providing a sufficient time is allowed for UX_1 , UX_2 to reach equilibrium with the uranium present, a period of about one hundred days. If one wishes to check the amount of uranium deposited by α counts, the density should not exceed 0.2 mg. of material per $cm.^2$. Uranium oxide can be electroplated from solution and the amount of uranium deposited determined by an α count of the resulting surface.⁹⁶ There are a total of 1480 α particles per minute or 740 with an upward component from a flat surface emitted per milligram of uranium. For every thousand α particles there are, under equilibrium conditions, about 490 β particles of UX_2 with a maximum energy of 2.32 m.e.v. peak energy. This ratio would be exactly 2:1 were it not for the α contribution of U^{235} . This hard UX_2 β radiation is accompanied, in addition to the α -rays, by the very soft β radiation of UX_1 and UY about equal in amount to the number of UX_2 β -rays. Care must be taken in using the above standard that α -rays and the soft β -rays do not enter the measuring instrument. Thin filters accomplish this purpose. Obviously, in using one of the above standards for determining the absolute activity of another substance, or the efficiency of a counter for measurement of other samples, it is necessary that the geometric conditions of sample distribution, etc., be the same in the two cases. Also, corrections for window thickness are usually necessary if the β -energy spectrum is significantly different for the two isotopes.

If the window thickness of the counter is known, this correction can be carried out as follows: Measure the counting rate of unknown and standard as a function of filtration by aluminum by interposing aluminum foils of known thickness. Plot the counting rate on the y axis of semilog paper as a function of filtration. The plot will, in general, be approximately a straight line except that, if the uranium standard is used with a thin window tube, there will be an upward break in the curve when filtration becomes so low that α and soft β particles enter the counting tube and are counted. The straight-line portion of each curve is extrapolated past the zero filtration point for a distance on the x axis corresponding to the window

thickness of the counting tube equivalent in aluminum. The ratio of this corrected counting rate for uranium or other standard to the known β activity of the standard represents the true counter efficiency for the standard, corrected for window absorption. It is now assumed that the unknown has been counted with the same efficiency after a similar window thickness correction is made, and the total β activity of the unknown is computed.

An additional precaution may be important. If the samples are backed by brass or other dense material, there will be considerable back-scatter of β -rays originally starting in a direction away from the counting tube. The fraction of the particles thus counted is dependent upon β energy. To minimize this effect the standard and sample may be deposited on cellophane or another light substance stretched on an embroidery hoop type of sample holder. Back-scattering metal can thus be removed several centimeters from the counting tube and back-scatter greatly minimized. As the average β -ray energy decreases, the experimental precautions necessary increase. Norris and Inghram⁹⁷ have described the procedure based on the above principle for determining the absolute activity of C^{14} samples.

Gamma radiation from an isotope such as I^{131} will introduce a small error unless correction is made. The increased counting rate over background remaining after filtration has removed all β activity will indicate the magnitude of this correction to be subtracted from the apparent β count.

As Evans points out,⁹⁸ one cannot directly calculate disintegrations per minute from β -activity determinations unless the disintegration scheme of the isotope in question is known. For example, the disintegration of Mn^{52} proceeds in only 35% of its atoms by positive β -ray emission. In the remaining cases, a K-orbit electron is absorbed, this reaction being accompanied only by Auger electron and x-ray emission. Some disintegrations give no β - or x-rays at all. In some instances γ radiation is strongly absorbed by the electronic structure of the disintegrating isotope giving many conversion electrons, which differ from nuclear β -rays in being essentially all of one energy.

A second method of calibrating for absolute sensitivity involves coincidence measurements of β - and γ -rays emitted from an isotope with a 1:1 correspondence of β -rays and γ quanta. The percentage of γ counts for which coincident β counts are recorded will also represent the efficiency of the counter in detecting all β -rays from the sample. Use of a β -ray spectrometer in combination with this technique of coincidence measurements has been developed as a powerful tool for ascertaining in an unambiguous

⁹⁶ D. E. Hull and B. Cohen, *Manhattan District Declassification Code 387* (Oct. 10, 1946).

⁹⁷ L. D. Norris and M. G. Inghram, *Phys. Rev.*, **73**, 350 (1948).

⁹⁸ R. D. Evans, *Nucleonics*, **1**, No. 2, 32 (Oct., 1947).

manner the disintegration schemes of positive and negative β -ray emitters.⁹⁹

X. PLANNING OF EXPERIMENTS

The most commonly accepted unit for measuring radioactivity in disintegrations per unit time is the *curie*. A curie is the number of disintegrations occurring in unit time per gram in pure radium. The commonly accepted value is 3.7×10^{10} disintegrations per second or 2.22×10^{12} disintegrations per minute. By general, although so far unofficial, extension a curie of any radioactive isotope is considered to be the amount in which 3.7×10^{10} disintegrations per second occur. The more commonly used subunit, the *millicurie*, equals 2.22×10^9 disintegrations per minute, and the *microcurie* equals 2.22×10^6 disintegrations per minute. A suggested new unit now also in use is the *rutherford*.⁹⁸ One rutherford, abbreviated "rd.," is the amount of any radioactive isotope which disintegrates at the rate of 10^6 disintegrations per second. In the common case in which each disintegration occurs through β -ray emission, the above figures represent the number of β -rays per unit time. The specific activity of any preparation of an element is the activity of the element in one of the above units per unit weight. A common unit is millicuries per gram of element (mc./g. element).

If one then has available knowledge concerning the total amount of radioactive isotope available and its specific activity, together with the amount of radioactivity and the sample size necessary for the analytical method it is proposed to use, then one usually has the raw material necessary for a calculation of the radioactivity that can be expected in crucial samples obtained during an experiment. It is well worth while planning experiments carefully on the basis of such calculations to insure that the required measurements can be made to an accuracy such that the desired conclusions may be drawn. The most common cause of failure of such phantom or real experiments is that the radioactivity is so diluted during the course of the experiment that radioactivity is not accurately measurable in the final fraction to be analyzed.

Pure C^{14} would contain approximately 5 millicuries of activity per milligram of carbon. As obtained from the Atomic Energy Commission at the present time, it is diluted with C^{12} , the most abundant normal isotope, to the extent that 1 mc. of activity is contained in about 20 mg. of carbon. Assuming the use of a gas chamber for measurement purposes so that 1000 disintegrations per minute per gram of carbon can be easily measured, one millicurie of C^{14} can be evenly distributed through $2.22 \times 10^9 / 1000 = 2.22$

⁹⁹ W. C. Peacock and M. Deutsch, *Phys. Rev.*, **69**, 306 (1946).

$\times 10^6$ grams, or approximately two tons of carbon and still be easily measured quantitatively. Thus, C^{14} containing carbon as received from Oak Ridge can be diluted by a factor of $2.22 \times 10^6/0.02 = 1.1 \times 10^8$ and still be quantitatively measured.

Yet even the ability to make measurements after these dilutions does not make feasible all otherwise potentially useful experiments, particularly in the biological field. For example, one might wish, by C^{14} labeling, to study the distribution of a potent biologically toxic material in man or animal, for example, botulinus toxin. This potent toxin has a molecular weight around 9×10^5 . From mouse toxicity data¹⁰⁰ it has been calculated that the lethal dose in man is about 0.1 μ g. If a synthesis were available allowing the incorporation of one atom labeled carbon per molecule of toxin, the labeled carbon content in a dose lethal to man would be:

$$(1 \times 10^{-7} \times 12)/(9 \times 10^5) = 1.3 \times 10^{-12} \text{ gram}$$

A 70-kilogram man would contain about 14 kilograms of carbon. Assuming an even distribution of a lethal dose of such a labeled material in man, the dilution factor will be:

$$(1.4 \times 10^4)/(1.3 \times 10^{-12}) = 1 \times 10^{16}$$

Comparing this dilution factor of 1×10^{16} with the attainable dilution factor of 1.1×10^8 calculated previously, we find that we have failed to design a practical experiment by the tremendous factor of 10^8 .

It is where high dilutions are necessary that tritium attains one potential advantage over C^{14} as a carbon label. Because of its much shorter half-life, one gram equivalent emits nearly 200 times as many β -rays per unit time as does C^{14} .

The radiations from radioactive isotopes have in themselves chemical and biological effects. Care must be taken that such effects do not invalidate the transference of the results of experiments to the more usual conditions where radioactivity is not present. Since it is during synthesis of radioactively labeled substances that radioactivity is usually most concentrated, it is here that the course of chemical reaction is most likely to be influenced by radioactivity.

In biological experiments, if concentrations of β -emitting radioactive elements higher than 0.01 microcurie per gram of tissue are reached for appreciable periods, the possibility of direct radiation effects should be considered in interpreting experimental data. Alpha emitters may produce biological effects invalidating tracer studies at 50-fold lower concentration.

¹⁰⁰ F. W. Putnam, C. Lamanna, and D. G. Sharp, *J. Biol. Chem.*, **165**, 735 (1946).

Many chemical compounds when labeled by radioactivity may be detected at far lower concentrations than by the chemical methods ordinarily used. At such low concentrations such effects as adsorption, coprecipitation, etc., may become many orders of magnitude more important than at usual concentrations. In the preparation of dilute standards for radioactive measurement one may be unaware of reaching such high dilutions that those effects invalidate the interpretation of experimental results. It has often happened that investigation showed more than 75% of a supposed radioactive standard adsorbed on the glass walls of a volumetric flask. Difficulties of this type in preparing dilutions of standards can be avoided by adding radioactively inert material chemically identical with the radioactive standard at each stage of dilution so that appreciable concentrations are preserved throughout the process.

Finally, if often happens that the limit of accuracy of experiments is set, not by the fundamental sensitivity and reproducibility of the physical and chemical procedures utilized, but instead by radioactive contamination in the laboratory. Useful procedures in avoiding such contaminations are:

(1) Isolate areas where highly concentrated or large amounts of radioactive preparations are handled as well as possible from regions where low-activity samples are measured or prepared for measurement.

(2) Classify glassware by some permanent marking system as to activity of preparation for which it is to be used. Carefully avoid use of any glassware for low-activity preparations once they have been used with highly radioactive material. This type of segregation can often be advantageously applied to tools and other apparatus. Clean and dry different classes of glassware separately and in a separate location. Do not use cleaning solution or brushes in common. Even electrodes for *pH* measurements can grossly contaminate low-activity solutions if previously used in solutions of high activity.

(3) To avoid contamination of measuring apparatus and area, keep the radioactivity of samples prepared for measurement at the same order of magnitude by diluting with inert material if necessary. If uncertain as to activity of an unknown, first check activity at a considerable dilution.

It will usually prove poor practice to avoid contamination difficulties by increasing the amount of radioactivity used in tracer studies. Unless other beneficial changes in procedure are made, the same difficulties will recur later. Moreover, the range of dilution over which tracer studies would otherwise have been feasible will have been reduced. For the same reason, other conditions being equal, it is advisable to use as sensitive a method as possible for the measurement of radioactive isotopes of long half-life.

XI. EVALUATION OF RADIATION HAZARDS TO PERSONNEL

Radiation hazards from radioactive materials may be divided into two classes, hazards from radiating sources external to the body, and hazards arising from radioactive materials taken into the body by absorption through the intact or broken skin, or by injection, swallowing, breathing, etc. The radiations from radioactive materials are peculiarly dangerous because of the long periods that may intervene between exposure and the first noticeable effects, thus allowing a high cumulative dosage before the danger is realized. A single dose of radiation to the skin sufficient to cause eventual complete desquamation of the epidermis may show no effect for ten days to two weeks. Long-continued exposure to radiation intensities too low to be acutely damaging may produce damage latent for long periods. Years after the last exposure, skin changes may develop which culminate in malignant growths fatal to the individual.

For β and γ radiation the evidence is fairly clear that the radiation dissipated per unit weight of a specified tissue measures the damaging ability of the radiation. Thus, as would be expected, the damaging effect to a tissue is measured by the energy absorbed by unit amount of tissue from the radiation beam rather than by the energy of the beam traversing the tissue. It has been found that, associated with the high density of ionization such as is produced in the neighborhood of an α particle, there is on many organisms a greater effect per erg of energy absorbed than for the more diffusely ionizing β and γ radiation. For protective measures this factor is usually taken as ten and the permissible dose in ergs per gram considered one-tenth of the permissible β and γ dose.

In the use of x-rays or γ -rays for therapy, dosage has long been measured in r -units or roentgens. For soft tissues one r -unit is the equivalent of about 93 ergs of energy in the form of x- or γ -rays absorbed per gram of tissue from the incident beam. The term r or roentgen is ordinarily restricted to x-ray and γ radiation. The term rep (roentgen equivalent physical) has come into use as a comparable unit for radiation dosage from ionizing particles. A gram of tissue is said to have received a dose of 1 rep of β radiation, γ radiation, etc., when it has absorbed from such an ionization radiation 83 ergs. This is the energy absorbed by one gram of air per r -unit.

The present average daily permissible or "tolerance" dose of β and γ radiation is usually given as 0.1 r or rep to the whole body or any portion thereof. Actually, so little is yet known about the long time effects of chronic irradiation that it is desirable to keep ordinary exposures below 0.01 rep.

1. External Radiation

Alpha rays will not, in general, penetrate the cornified layer of the epidermis, and therefore are not an important hazard from sources external to the body. Since β -rays from radioactive sources will at most penetrate only a few millimeters of tissue, damage from external β sources will usually be confined to the skin. Almost certainly, however, exposure of any one area to β -rays should not exceed an average of 0.1 rep daily.

If UX_2 , a β emitter with a maximum β energy of 2.3 m.e.v., is spread uniformly over a flat surface at a concentration of one microcurie per cm^2 , the radiation dose to human skin in contact with this surface is about 2.8 rep per hour. If one microcurie of this same material, UX_2 , is concentrated as a point source, the radiation dose at 1 cm. distance is about 0.45 rep per hour. These figures are typical only of hard β emitters, the tissue dosage decreasing with reduced energy.

The γ radiation from one millicurie of radium in equilibrium with its disintegration products and shielded with 0.5 mm. of platinum is 8.4 r per hour at a distance of 1 cm. from a point source.¹⁰¹ The γ radiation from unshielded Na^{24} , where each disintegration is accompanied by two γ quanta, has about the same intensity. The intensity of β radiation from Na^{24} is some fifty times greater.

One notes that even the relatively small amounts of radioactive isotopes used in tracer work can be hazardous to the investigator through external exposure. The greatest hazard to the chemist is to the hands in handling concentrated sources, since the inverse square law usually reduces exposure to the rest of the body several fold. The pencil ionization chambers and photographic films used in personnel monitoring, if worn on the chest, usually underestimate by many times the exposure of hands and face if radioactive preparations emitting hard β - and γ -rays are handled without precaution. Reliance should not be placed on them to indicate complete safety. Rather it is essential that the quantitative hazards of the procedures it is proposed to use be thoroughly evaluated.

2. Internal Radiation

For details concerning the very real hazard that may exist from radioactive materials introduced into the human body either intentionally in tracer experiments or by accident, the extensive data originating in the Manhattan Project should be consulted, as it appears in declassified docu-

¹⁰¹ T. N. White, L. D. Marinelli, and G. Failla, *Am. J. Roentgenol. Radium Therapy*, **44**, 889 (1940).

ments, in the current journal literature, or in project monographs.^{102, 103} Marinelli and his associates^{104, 105} have given the quantitative methods for calculating the dosage due to active isotopes provided the internal distribution in the body is known. The average β energy for many radioactive isotopes is given by Marinelli, Brinckerhoff, and Hine.¹⁰⁶ The same method can be applied to α -emitting isotopes except that, due to the greater specific ionization mentioned earlier, the biological effect per unit ionization dose is greater by perhaps ten times. Evans¹⁰⁷ has reviewed the methods useful in determining radium content of the body in cases of suspected radium poisoning as well as the probable lethal dosage.

3. Personnel Monitoring

A. INSTRUMENTATION

A considerable variety of portable ionization-measuring instruments are now commercially available for personnel and laboratory monitoring, as well as badges containing calibrated, radiation-sensitive photographic film. Ordinarily such film is returned to the distributor for processing and evaluation of the radiation dose.

Portable G-M counters because of their high sensitivity are excellent for qualitative detection of hard β and γ emitters. The dependence of the calibration of such units on the quantum energy of the radiations they measure is such that readings in general survey work often have only semi-quantitative significance.

Ionization chamber type instruments often have good γ -ray calibration. Calibration for β radiation is much more approximate and likely to be low by a factor of two or three if the γ -ray calibration is used. It is always advisable to check routinely the calibration of portable instruments by radioactive standards rather than depending upon the original calibration being retained as batteries run down and tubes and other components change with age or use.

General rules and precautions governing the use of radioactive materials in some of the Atomic Energy Commission laboratories have been published.¹⁰⁸

¹⁰² *National Nuclear Energy Series* (Manhattan Project Technical Section), to be published.

¹⁰³ K. Z. Morgan, *J. Phys. and Colloid. Chem.*, **51**, 984 (1947).

¹⁰⁴ L. D. Marinelli, *Am. J. Roentgenol. Radium Therapy*, **47**, 210 (1942).

¹⁰⁵ L. D. Marinelli, E. Quimby, and C. J. Hine, *Nucleonics*, **2**, No. 4, 56 (April 1948).

¹⁰⁶ L. D. Marinelli, R. F. Brinckerhoff, and C. J. Hine, *Revs. Modern Phys.*, **19**, 25 (1947).

¹⁰⁷ R. D. Evans, *Am. J. Roentgenol. Radium Therapy*, **37**, 368 (1937).

¹⁰⁸ Clinton Laboratory Staff, *Nucleonics*, **1**, No. 4, 60 (Dec. 1947).

B. BLOOD STUDIES

One of the earliest signs of damage from acute or semiacute radiation exposure is the destruction or alteration of the formed elements and particularly the white cells of the blood. Therefore, routine blood studies including differential counts are advisable in checking whether such acute generalized exposure has occurred. Unfortunately, the usual blood studies are not a reliable means of ascertaining damage of a more chronic type due to exposure extending over months or years. Evidence obtained from experimental animals¹⁰³ indicates that radiation may induce a very significant shortening in life, as well as sterility and lowered vigor, without significant changes being detectable in the circulating blood by the usual blood study methods. Therefore, reports of normal blood counts emphatically should not be taken to indicate that dangerous overexposure to radiation is not occurring.

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MASS SPECTROMETRY

DAVID W. STEWART, *Eastman Kodak Company*

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I. INTRODUCTION

Mass spectrometry deals with the separation of gaseous ions of differing mass by the action of electric and magnetic fields, and with the measurement of the abundance of the various ionic species revealed by this separation. The mass spectrometer thus identifies isotopes, measures their relative abundances, and makes possible tracer work with separated stable isotopes. From more complex compounds it usually forms ions by dissociation as well as by simple ionization, and gives a spectrum of masses which is characteristic of the compound for each set of ionizing conditions. If a mixture is analyzed, the resulting spectrum often yields a qualitative analysis by inspection, and a quantitative analysis may be made if calibrating spectra of the pure components are available. The wartime use of mass spectrometers, not only as control instruments in the synthetic rubber, aviation gasoline, and atomic energy programs, but also as actual production devices for the separation of large quantities of U^{235} , has greatly accelerated the development of instruments and techniques in this field.

Despite uses which may be classed as routine, mass spectrometers cannot yet be regarded as commonplace pieces of laboratory equipment nor is the detailed theory of their operation completely understood. The organic chemist wishing to apply the technique is at present offered either the choice of a limited number of commercial instruments, at prices ranging from approximately \$10,000 to \$35,000, or the problem of constructing an instrument to meet his own requirements. In either case, the difficulty of the chemist is not over when the apparatus has been installed and operators have been trained for its routine use. Experience has shown that the adjustment, maintenance, and particularly the adaptation of the equipment to new problems will require as a minimum the part-time help of someone skilled in electronic and high-vacuum techniques. For maximum effect

tiveness, especially in applications not yet reduced to a routine basis, the mass spectrometer will require a full-time research man to supervise its operation and assist in the interpretation of results. Because of personnel requirements and the high initial cost of most installations, the use of the mass spectrometer is still largely limited to those laboratories able to utilize it on a full-time basis. In many industrial applications, the spectrometer must compete with conventional methods of analysis, and economies can be effected only if a considerable number of samples are run each day. However, from the progress which has been made in instrument design in the last half dozen years, and from the increasing number of installations—each of which, in turn, contributes new applications and techniques—it is clear that the field of mass spectrometry is of growing importance to the organic chemist.

The basic literature on mass spectrometry was written largely for research physicists, and chemists have had little opportunity to become familiar with the subject and its possible applications. The present chapter is designed to provide this information. Apparatus of various general types for use in organic chemistry, and factors affecting its design, operation, and maintenance, will be described and applications and limitations of the method will be emphasized. Details of design and use of specific commercial mass spectrometers are still confined largely to manuals issued with the instruments.

II. PRINCIPLES OF THE METHOD

1. Mass Spectrographs of Thomson and Aston

When the atoms or molecules of a substance present as a gas at very low pressure are subjected to bombardment by electrons from a heated filament or other source, both positively and negatively charged ions are produced. If the electrons are sufficiently energetic, they will not only produce molecular ions but will also break chemical bonds within the molecule. Some of the molecular fragments may be neutral, others will be positive ions having a deficiency of one, two, or more electronic charges, while still others may have excess electrons and be negative ions. The process of ionization by electron impact is discussed further on page 2034. If the pressure is low enough, the ions may travel over lengthy paths before regaining their neutral states by collisions with other molecules or with the walls of the container. While ionized, these gas molecules and fragments may be caused to move by applying an electric field. If such a field is produced between successive parallel plates, positive ions moving toward the more negative plates can be collimated into a beam by suitable defining slits. If the residual gas pressure is low enough so that only a negligible

number of collisions take place, and in the absence of any other field, this beam will continue in a straight line, and its presence may be detected by its effect on a photographic plate or by the electrical effect of its net positive charge.

Such a beam of ions, like a current flowing in a wire, is deflected by any magnetic field which crosses its direction of flow. If the field is at right angles to the motion, the deflection, y , of an individual ion is proportional to the strength of the magnetic field, H , and the ionic charge, e , and is inversely proportional both to the mass, m , and the velocity, v , with which the ion moves. Thus:

$$y = k(He/mv) \quad (1)$$

If the moving ion is influenced by an electrical field of strength E , in a direction also perpendicular to its motion, it will be deflected independently a distance, x , given by:

$$x = k'(Ee/mv^2) \quad (2)$$

where k and k' are constants of the apparatus.

In the spectrograph devised by J. J. Thomson,¹ a beam of ionized gas molecules was passed through electric and magnetic fields arranged in the same direction so as to make deflections x and y lie at right angles to each other in the plane of a photographic plate. When the gas contained only one species of ion, so that e/m was a constant, a blackened trace was found on the developed photographic plate indicating the locus of impact of ions of varying velocities. From the equations above (for a given value of e/m) this locus can be seen to have the form of a parabola:

$$y^2/x = \text{constant} \quad (3)$$

When ions of different masses were present, a number of separate parabolas were observed. The parabolas corresponding to various masses could be identified, and e/m measured by their displacements, while the relative abundance of each ion could be estimated by the blackening produced in the photographic emulsion.

The method of parabolas, while useful in demonstrating the presence of isotopes, was inconvenient for obtaining either exact measurements of e/m or abundance measurements of particular ions. An improved mass spectrograph, with better resolution and higher intensity, using the same principles of electric and magnetic deflection but a different arrangement of fields, was designed by Aston.² With the x and y deflections separated by 180° instead of 90° , ion beams for various values of e/m were focused at

¹ J. J. Thomson, *Phil. Mag.*, 21, 225 (1911).

² F. W. Aston, *Phil. Mag.*, 38, 707 (1919).

points along a straight line, with the position of focus a measure of the mass-to-charge ratio, and the blackening of the plate a measure of the abundance.

2. Mass Spectroscopy and Mass Spectrometry

Following Aston, work in positive-ray analysis began to follow two separate courses. One group of workers was primarily interested in the exact measurement of the masses of ions. Ingenious equipment of great precision has been designed for this work and values of atomic weights may be obtained with an accuracy of a few parts per million. In this branch of mass analysis, known as mass spectroscopy, photographic recording is still used as the most accurate measure of a displacement length, and the instrument employed is called a *mass spectrograph*. The organic chemist is rarely concerned with this type of investigation. The other type of information available from a mass spectrum is the relative abundance of the various ionic species, and the measurement of these abundances is called *mass spectrometry*.

3. Dempster Mass Spectrometer

At almost the same time as Aston's work, Dempster² devised an instrument particularly suitable for making abundance measurements. In this apparatus, equal kinetic energies were given the various ions by uniform acceleration, and the beam was then analyzed by deflection through 180° in a magnetic field. No electrostatic deflection was used. This apparatus is shown schematically in figure 1. Details of the ion source will be discussed later, but for the present it may be assumed that two species of gaseous ions have been formed having masses m_1 and m_2 (in grams), and that each ion has a single positive charge, corresponding to the loss of one electron of charge e . If all the ions are uniformly accelerated by an applied voltage of E (in electrostatic units), the energy acquired by each will be given by:

$$Ee = \frac{1}{2} m_1 v_1^2 = \frac{1}{2} m_2 v_2^2 \quad (4)$$

where v_1 and v_2 are velocities in centimeters per second.

The velocity of each may be expressed in terms of the mass and accelerating potential as:

$$v_1 = (2Ee/m_1)^{1/2} \text{ and } v_2 = (2Ee/m_2)^{1/2} \quad (5)$$

² A. J. Dempster, *Phys. Rev.*, **11**, 316 (1918).

In the magnetic field, the particles will be deflected in a series of arcs whose radii, r_1 and r_2 (in centimeters), are given by:

$$m_1 v_1 = H e r_1 \text{ and } m_2 v_2 = H e r_2 \quad (6)$$

where H is the uniform magnetic field strength (in gaussess). Substituting v 's as defined by equation (6) in equation (4) gives:

$$E e = H^2 e^2 r_1^2 / 2 m_1 = H^2 e^2 r_2^2 / 2 m_2 \quad (7)$$

whence:

$$r_1 = \frac{1}{H} \left(\frac{2 E m_1}{e} \right)^{1/2} \text{ and } r_2 = \frac{1}{H} \left(\frac{2 E m_2}{e} \right)^{1/2} \quad (8)$$

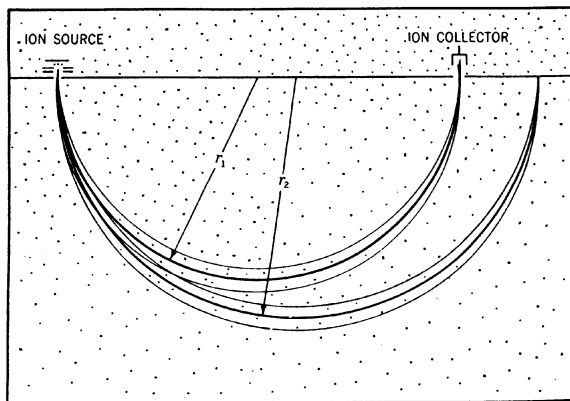


Fig. 1.—Magnetic focusing in the Dempster 180° mass spectrometer.

When all quantities are expressed in practical units, H in gaussess, E in volts, r in centimeters, and m in atomic weight units ($O^{16} = 16$), this becomes:

$$H r = 144.00 (E m)^{1/2} \quad (9)$$

With constant values for H and E :

$$r_1 / r_2 = (m_1 / m_2)^{1/2} \quad (10)$$

In figure 1, ions of mass m_1 are shown focused at the ion collector, while those of mass m_2 are brought to a focus at some distance. Ions leaving the last collimating slit in the ion source retain a divergence of direction which depends in magnitude on the width of the slits used and on the distance

separating successive collimators. In order to allow as many ions as possible to be accelerated, slits are made as wide as the necessary resolution will permit. If α is the total angle of divergence, ions leaving the source at an angle of $+\alpha/2$ will travel outside the normal path, while those leaving in a direction of $-\alpha/2$ will travel on an inside path, but the radii of curvature for all ions of specified mass and charge will be the same. For small values of α , as illustrated in figure 1, the divergent paths having a common radius will converge after a deflection of 180° , to produce an image of the source exit slit. Ions differing only slightly in mass will thus be resolved only at the end of the semicircular path. By changing either the accelerating potential, E , or the magnetic field strength, H , as indicated in equation (8), the radii of other ion paths may be adjusted to focus at the collector slit, and the abundance of ions of different masses measured successively.

In the Dempster type of instrument, individual ion beams are allowed to fall on an insulated collector, and the net positive charge, leaking to ground through a high resistance, creates an e. m. f. which can be measured and which is proportional to the number of ions being collected per second. Photographic recording could also be used, with the added advantage that extremely rare ionic species could be made detectable by extended exposure. However, the blackening effect of ions on a photographic plate is only an indirect measure of abundance, since their photographic effectiveness is undetermined, and depends upon a number of factors. For this reason, it is usually more accurate and convenient to measure abundances by direct electrical methods. The design of the 180° mass spectrometer most generally used is that described by Nier⁴ in 1937.

4. Sector-Type Mass Spectrometer

Barber⁵ and Stephens⁶ have shown that if a divergent beam of ions enters and leaves a uniform magnetic field normal to the pole edges, the ions are brought to a focus on a line extended from the source through the center of curvature of the ion beam. The 180° magnetic refocusing already described is a particular case of this type. When the magnetic field is produced between sector-shaped pole pieces, which may be truncated as shown in figure 2, and which may be of any convenient angle (60° , as shown in the figure, and 90° have been commonly used, but the resolution is independent of this angle), refocusing is obtained when the field strength and accelerating voltage are adjusted so that the central ray of ions enters and leaves in a direction normal to the edges, and hence has its center of curvature at the

⁴ A. O. Nier, *Phys. Rev.*, **52**, 933 (1937).

⁵ N. F. Barber, *Proc. Leeds Phil. Lit. Soc., Sci. Sect.*, **2**, 427 (1933).

⁶ W. E. Stephens, *Phys. Rev.*, **45**, 513 (1934).

apex of the sector. In this arrangement, ions are produced outside the magnetic field, reach it after traveling some distance in a straight line, pass through a region of nonuniform fringing flux, and then enter the homogeneous magnetic field where the main deflection occurs. The path is repeated in reverse from the magnet to the collector. A number of advantages have been claimed for this arrangement: (1) The area of the magnetic field for a given radius of curvature is reduced, thus requiring a less massive magnet and less power; the use of a permanent magnet also becomes possible. (2) With the ion source located outside the field, the spacing between the magnet pole pieces may be made narrower (again reducing power consump-

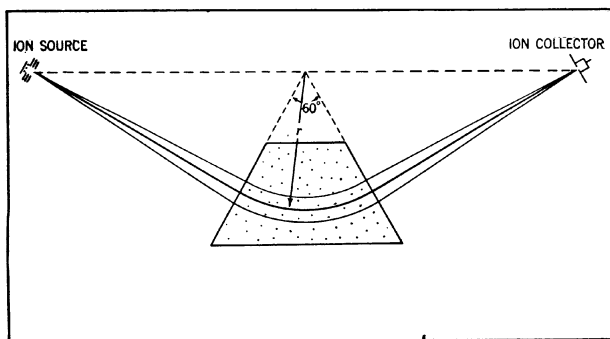


Fig. 2.—Magnetic focusing in the sector-type mass spectrometer.

tion for any required field strength), since now only the ion beam and its envelope must be accommodated between the poles. (3) The removal of the ion source to a field-free region has been suggested as desirable since discrimination between ions may occur if a magnetic field is present in the ionizing region (see page 2015).

Disadvantages are equally obvious: (1) The ions must travel an additional distance to and from the magnetic analyzer, and thus may suffer additional scattering from collisions with residual gas molecules. (2) The greater distance from source to receiver increases the difficulty of maintaining mechanical alignment. (3) Most ion sources utilize a magnetic field to align the beam of ionizing electrons, and in the sector type this must be provided by an auxiliary magnet at the source; when this arrangement is used, advantage (3) is not only nullified but further variable discrimination is introduced because of the nonuniform nature of the usual auxiliary field. (4) The sector-shaped field, with regions of fringing flux, does not have accurately located borders and an apex which may be used for geo-

metrical alignment of the source and receiver. Coggeshall⁷ has shown how appropriate corrections can be calculated, but these are subject to increasing uncertainty as the angle of divergence of the beam increases.

From a practical standpoint, it should be pointed out that either the sector or the 180° instrument can probably be made to give results of any required degree of accuracy. Scattering in either type can be minimized by operation of the analyzer at a pressure where the mean free path of the ions is many times greater than their geometric path length. Experimentally, the source and receiver of the sector instrument can be accurately aligned during construction and the assembly then located in the magnetic field with no more elaborate tools than a millimeter scale and try-square. Final minor adjustments, to give maximum ion intensities and resolution equal to the theoretical for the slit widths and radius of curvature used, can then be made with the instrument in operation. By utilizing a design in which the analyzer is integral with the pole pieces,^{8, 9} even this adjustment could presumably be eliminated.

The resolving power of the spectrometer, which is defined as the highest mass, m , which can be separated from a peak Δm mass units away is given by the expression:¹⁰

$$\frac{m}{\Delta m} = \frac{\text{Radius of curvature}}{(\text{width of entrance slit}) + (\text{width of collector slit})} \quad (11)$$

This applies to all direction-focusing instruments and is independent of the angle of deflection.

5. Double-Focusing Mass Spectrographs

Equation (4), for the separation of ions of mass m_1 and m_2 in spectrometers using only magnetic dispersion, requires that all ions have identical kinetic energies when they leave the ion source. If positive ions are formed at rest, without initial kinetic energy, and at points where the potential is uniformly $+E$, then this condition will be satisfied when the ions have been accelerated through the last collimating slit, which is usually at a potential of zero. However, if the ions are formed at points where the potential may be $E \pm \Delta E$, or have varying amounts of initial kinetic energy, then ions with a corresponding spread in mass, $m \pm \Delta m$, will be brought to focus simultaneously, and poor resolution will make the instrument unusable. The first of these difficulties may be largely overcome by forming the ions in a region of low potential gradient, and the second effect is not serious if the sample can be vaporized and ions obtained by electron bombardment. While initial kinetic

⁷ N. D. Coggeshall, *J. Applied Phys.*, **18**, 855 (1947).

⁸ N. D. Coggeshall and E. B. Jordan, *Rev. Sci. Instruments*, **14**, 125 (1943).

⁹ J. A. Hipple, U. S. Pat. 2,417,797, to Westinghouse Elec. & Mfg. Co. (March 18, 1947).

¹⁰ H. G. Thode, R. L. Graham, and J. A. Ziegler, *Can. J. Research*, **B23**, 40 (1945).

energies present under such conditions contribute to some discrimination in the ion source and tend to lower the resolution, nearly all of the present applications of mass spectrometry to organic chemistry have been achieved with instruments of the types already described. If future work, especially with nonvolatile organic substances, requires special ion sources and special processes of velocity selection, a number of double-focusing mass spectrographs are available for this purpose.¹¹⁻¹⁵ Such instruments utilize a combination of electrostatic and electromagnetic deflections so arranged that the final dispersion of ions is independent of initial energies. A review of the most important types of double-focusing instruments is given by Inghram.¹⁶

The Mattauch type of instrument has received extensive development^{17,18} in recent years because of the large range of masses which can be resolved on a single photographic plate. An a.-c. operated instrument of this type, which has been used for chemical analysis studies of inorganic materials, as well as for isotopic abundance work and the exact measurement of atomic masses, has been described recently by Shaw and Rall.¹⁹

6. Other Types of Mass Spectrometers

At least two special mass spectrometers have been described which differ so radically from the instruments thus far considered that mention should be made of them, even though their development is still in an early stage. Since neither instrument uses a magnetic field, it would appear that a considerable simplification of apparatus should be possible through their use if present limitations can be overcome.

In the Ion Velocitron²⁰ an ion source is used in which monoenergetic ions are emitted in pulses of microsecond duration. The ions travel, *in vacuo*, through a long, straight tube. At the opposite end, the time interval between the arrival of ions of mass m_1 and m_2 will be proportional to:

$$L[(m_1)^{1/2} - (m_2)^{1/2}]$$

where L is the length of the tube. The difference in arrival time may be observed on the screen of an oscilloscope, where the complete mass spectrum will be traced for each pulse. Limitations, thus far, are largely connected with poor resolution, low ion-beam intensity, and changes in the pattern with time.

¹¹ F. W. Aston, *Proc. Roy. Soc. London*, **A115**, 487 (1927); **A163**, 391 (1937).

¹² A. J. Dempster, *Proc. Am. Phil. Soc.*, **75**, 755 (1935).

¹³ K. T. Bainbridge and E. B. Jordan, *Phys. Rev.*, **50**, 282 (1936).

¹⁴ J. Mattauch and R. Herzog, *Z. Physik*, **89**, 786 (1934).

¹⁵ J. Mattauch, *Phys. Rev.*, **50**, 617 (1936).

¹⁶ M. G. Inghram, "Modern Mass Spectroscopy," in *Advances in Electronics*. Vol. I, L. Marton, ed., Academic Press, New York, 1948.

¹⁷ C. Reuterswärd, *Arkiv Mat. Astron. Fysik*, **A30**, No. 7 (1944).

¹⁸ H. Ewald, *Z. Naturforsch.*, **1**, 131 (1946). A. Klemm, *ibid.*, **1**, 137 (1946).

¹⁹ A. E. Shaw and W. Rall, *Rev. Sci. Instruments*, **18**, 278 (1947).

²⁰ W. E. Stephens, *Phys. Rev.*, **69**, 691 (1946). A. E. Cameron and D. F. Eggers, Jr., *Rev. Sci. Instruments*, **19**, 605 (1948).

In the Radio-Frequency Mass Spectrometer,²¹ ions are generated at the center of a series of four or more concentric grids in a suitable vacuum tube. A radio-frequency voltage is applied to the second grid, d.-c. voltages to the first and third grids, and a blocking voltage to the fourth grid. A collector plate is located on the outside. Ions of proper mass and velocity are accelerated from grid 2 to grid 3, and ultimately reach the collector, if they arrive at the accelerating electrode at the proper phase. A scan over the mass spectrum is accomplished by varying the radio frequency. With d.-c. voltages below 100 v. and an a.-c. voltage of less than 5 v., the frequencies required to scan from below mass 1 to mass 250 will vary from 3300 kilocycles to 104 kilocycles per second. This device was originally used to study negative ions, but it is likely to be useful for positive ions when its resolution is adequate or when the high cost of other spectrometers is not justified. Difficulties resulting from poor resolution have been minimized by using a two-stage tube with nine electrodes, with radio-frequency voltages on the third and sixth grids. Actual applications have not yet been reported.

7. High-Intensity Spectrometers for Separation of Isotopes

The instruments so far described, and indeed any that are likely to be directly useful in chemistry, serve to separate only unweighable quantities of material. The ion beams usually represent electrical currents of from 10^{-8} to 10^{-14} amperes. If such a current is carried by singly charged ions, Faraday's law permits one to calculate the amount of the substance deposited at the collector in unit time. For example, if nitrogen is being analyzed, and a current of N_2^+ ions of 10^{-8} amp. is obtained, it will require:

$$(96,500 \text{ amp. sec.}) / (10^{-8} \text{ amp.}), \text{ or } 9.65 \times 10^{12} \text{ seconds}$$

to deposit one gram equivalent, or 28 grams of nitrogen. Since this is more than 300,000 years, it becomes obvious that the analytical spectrometer is not adapted to the production of any quantity of separated material.

In the large-scale separation of the isotopes of uranium, which was accomplished first by the electromagnetic process (in a special type of mass spectrometer called a Calutron), many special problems connected with the production and focusing of high-intensity ion beams were successfully solved. Although this achievement is not of direct concern in the present chapter, it must be realized that a large amount of fundamental information has been obtained regarding mass spectrometry, and many useful techniques have been developed. Although for security reasons, much of the information may be released only after a long period of time, if at all, enough has been published to indicate that the ultimate effect on analytical mass spectrometry may be very great. Present information on the electromagnetic separation process is summarized by Smyth.²² Some possible applications of the Calutron to elements other than uranium have been published by Keim.²³

²¹ W. H. Bennett, *Instruments*, **22**, 38 (1949); also *Natl. Bur. Standards U. S. Tech. News Bull.*, **32**, 105 (1948).

²² H. D. Smyth, *Atomic Energy for Military Purposes*. Princeton Univ. Press, Princeton, 1945, Chapter XI.

²³ C. P. Keim, *Chem. Eng. News*, **25**, 2624 (1947); *Science*, **108**, 541 (1948).

Descriptions of high-intensity mass spectrometers developed outside the Manhattan District have been published.²⁴ An apparatus described by Koch and Bendt-Nielsen²⁵ is said to have a resolving power better than one mass unit in 238 with ion beams of 10^{-5} amp. Another mentioned by Mott²⁶ as having been developed in Germany yielded up to 0.5 mg. of product per 24 hours. A series of papers by Walcher²⁷ discusses space charge effects and suitable ion sources for spectrometers of the high current density type.

III. APPARATUS AND GENERAL TECHNIQUE

1. General Description—Availability

Mass spectrometers for general analytical purposes are commercially available in the United States, at present, from at least three sources—the *Consolidated Engineering Corporation*, Pasadena, California, the *General Electric Company*, Schenectady, New York, and *Process and Instruments*, Brooklyn 22, New York. In Great Britain, *Metropolitan-Vickers Electrical Co., Ltd.*, Manchester, issued provisional specifications for a general-purpose mass spectrometer in February, 1947, but it is not yet known what applications have been made of this instrument. For the detection of leaks in vacuum equipment—a specialized application of the mass spectrometer to be described briefly on page 2056—devices are available from a number of other American manufacturers, but details will not be discussed here.

Aside from leak detectors, instruments made by *Consolidated* are of two types: (1) a 180° instrument designed primarily for gas analysis of complex mixtures but also useful for isotope work, illustrated in figure 3; and (2) a 60° sector-type spectrometer, designed by A. O. Nier, of the University of Minnesota, and especially applicable to the analysis of isotopic mixtures. The *General Electric* all-purpose instrument is of the 60° sector type, with high resolution, and is stated to have applications paralleling those mentioned above for the 180° instrument. *Process and Instruments* is offering a 60° spectrometer for general use up to mass 100 (Fig. 4), as well as custom-built models of other types and separate components for those who wish to assemble their own apparatus.^{27a} Figure 5 shows a 60° tube ready for assembly in the spectrometer. The *Metropolitan-Vickers* design utilizes a 90° sector magnetic field but otherwise appears very similar to the *General Electric* instrument. All the instruments are equipped with electronically regulated power supplies for stable operation from a.c.

²⁴ W. R. Smythe, L. H. Rumbaugh, and S. S. West, *Phys. Rev.*, **45**, 724 (1934). R. H. Bernas and A. O. Nier, *Rev. Sci. Instruments*, **19**, 895 (1948).

²⁵ J. Koch and B. Bendt-Nielsen, *Kgl. Danske Videnskab. Selskab Mat. fys. Medd.*, **21**, No. 8, 28 pp. (1944).

²⁶ N. F. Mott, *Nature*, **158**, 861 (1946).

²⁷ W. Walcher, *Z. Physik*, **121**, 719 (1943); **122**, 62, 401, 604 (1944).

^{27a} *Rev. Sci. Instruments*, **19**, 124 (1948).

lines, and all except the *Consolidated-Nier* and the *Process and Instruments* models have recorders which automatically trace the intensity of ion peaks as various masses are brought to focus on the receiver.

In 1946 Coggeshall²⁸ estimated the initial cost of a complete mass spectrometer laboratory at \$20,000 to \$30,000. In 1948, the American instru-

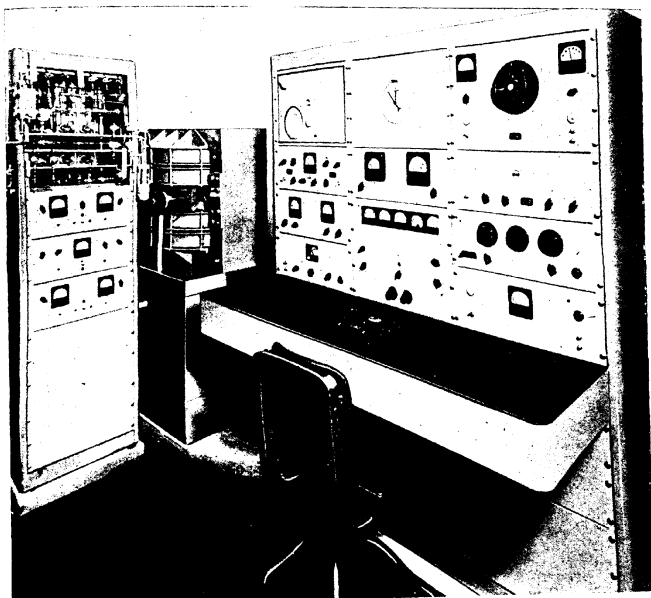


Fig. 3.—A commercial installation of the mass spectrometer (courtesy *Consolidated Engineering Corp.*, Pasadena, Cal.). In the center background are the magnet and spectrometer proper; to the left are the sample inlet system and evacuation apparatus; in the right foreground is the control table, containing recording apparatus and power supplies.

ments with automatic recorders sold at prices above \$30,000 for a complete installation in the user's laboratory, including the training of technicians in instrument theory, operation, and maintenance, but not including separate sample blending equipment or computers. The *Consolidated-Nier* spectrometer was described in June, 1947,²⁹ and became available commer-

²⁸ N. D. Coggeshall, *Chem. Inds.*, **58**, 420 (1946).

²⁹ A. O. Nier, *Rev. Sci. Instruments*, **18**, 398 (1947).

cially with certain engineering modifications³⁰ in 1948 at a price of approximately \$12,000. The *Process and Instruments* spectrometers are also in the \$10,000 to \$15,000 range. The mass spectrometer and recorder units

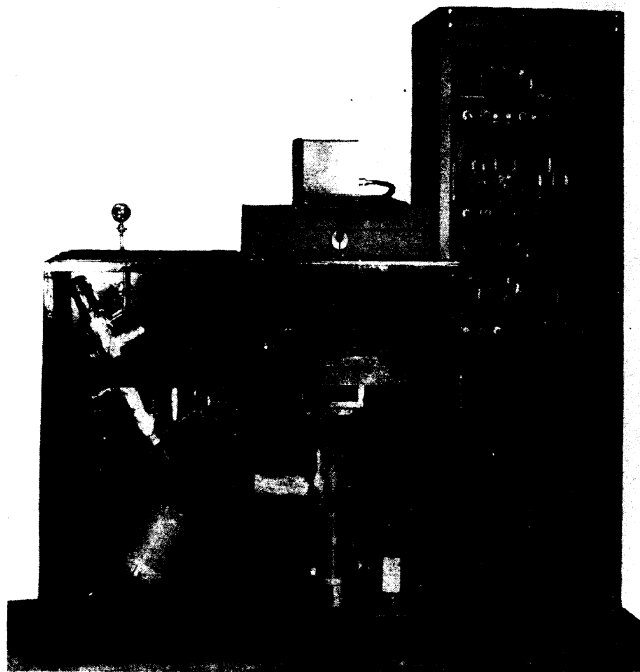


Fig. 4.—Rear view of 60° mass spectrometer (courtesy *Process and Instruments*). The unit at the left contains the spectrometer tube, magnet, and vacuum system. Electronic units at the right supply regulated power to the magnet, filament, ion-accelerating plates, and ion-current amplifier.

previously manufactured by the *Westinghouse Electric Corp.*, Baltimore, Maryland,³¹⁻³³ were withdrawn from the market in November, 1946.

³⁰ H. W. Washburn, *U. S. Naval Med. Bull. Supplement*, Mar.-April, 1948, p. 60.

³¹ J. A. Hipple, *J. Applied Phys.*, **13**, 551 (1942).

³² J. A. Hipple, *Electronics*, **16**, 120 (1943).

³³ J. A. Hipple, D. J. Grove, and W. M. Hickam, *Rev. Sci. Instruments*, **16**, 69 (1945). Also D. J. Grove and J. A. Hipple, *ibid.*, **18**, 837 (1947).

Although the trend is toward the use of commercial instruments, the large initial cost has led some laboratories to construct their own instruments. This has been especially true among those wishing to use tracers and not requiring the refinements necessary for gas analysis. Estimates of current construction costs are not available for a simple type of instrument suitable for isotope measurements on the lighter elements. In 1942,

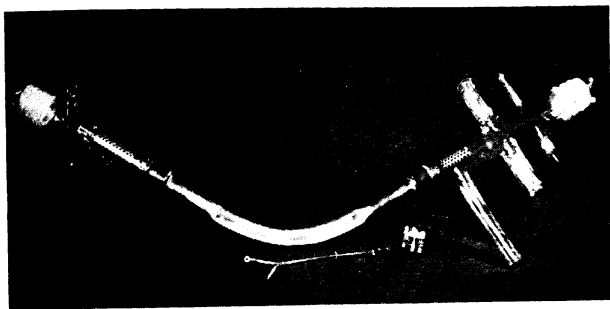


Fig. 5.—Mass spectrometer tube for 60° instrument (courtesy *Process and Instruments*). The glass envelopes are provided with removable caps for easy access to source (right) and receiver (left), and are attached to the copper analyzer tube by glass-to-metal seals. The ion source and filament stem are shown in the foreground before mounting on the source end of the analyzer.

however, Pollard and Davidson³⁴ estimated an expenditure of \$1100 for parts alone.

The general layout of a mass spectrometer is illustrated in figure 6, which happens to be for a 180° instrument, but which will serve schematically for other types as well. There are six essential parts of the apparatus. (1) A sample handling system is required, through which gases or volatile liquids can be introduced for analysis. (2) Associated with this are a capillary or orifice type of leak to admit a small fraction of the sample to the ion source, pressure-measuring devices, and pumps for evacuating the spectrometer tube and the inlet system. (3) The ion source consists of the filament for producing an electron beam, and electrode plates with suitable slit systems for accelerating and collimating the ionizing electrons and the ions which are to be analyzed. (4) The magnet, producing a uniform magnetic field perpendicular to the plane of the figure, is not shown, but the analyzer tube, including source and collector in the 180° type, lies between

³⁴ E. Pollard and W. L. Davidson, *Applied Nuclear Physics*. Wiley, New York, 1942, p. 178.

pole pieces which produce the necessary dispersion of masses. (5) At the opposite end of the analyzer tube from the source, the ion collector may be of the single-slit type shown or it may permit the simultaneous collection of two beams of ions. (6) The measurement of collected ion currents depends on electronic amplifiers, which in turn may be coupled to output

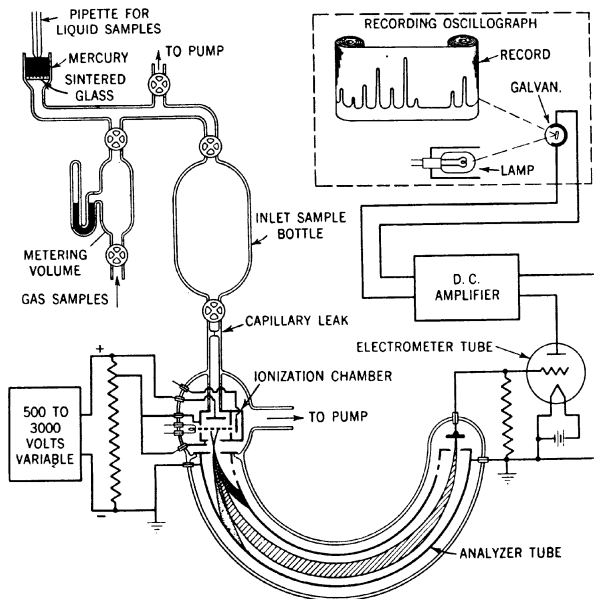


Fig. 6.—Schematic diagram of mass spectrometer and associated equipment (courtesy Consolidated Engineering Corp.).

meters of various types or to a recorder, either of the photographic type shown or one using a traveling pen.

For proper arrangement of one of the commercial mass spectrometers and associated equipment, including a gas-blending system for the preparation of standard samples, a room at least 12 feet by 16 feet has been recommended. For proper stability of the electronic circuits, it is desirable that this be air conditioned at about 70° F. and not more than 50% relative humidity. Electrical supply circuits for the electronic components should be as free as possible from voltage fluctuations and may require preliminary regulation. Mechanical vibration is undesirable for the electronic tubes as well as for galvanometers and should be avoided when pos-

sible, but shock-absorbing mountings have been applied successfully so that operation is possible even very near heavy engines or other sources of vibration. Suitable galvanometer mountings are discussed by West in Chapter XXI. A supply of cooling water for diffusion pumps and a drain are generally required.

2. Sample-Handling Systems

When it is unnecessary to measure accurately the pressure of gases to be analyzed, as in making isotopic ratio determinations, the sample manifold may be reduced to a very simple form. Samples are usually contained in glass bulbs with stopcocks and standard-taper ground joints. These may be attached to an inlet system which is isolated from the leak to the spectrometer by a stopcock. The space between the sample bulb and this stopcock is evacuated by a diffusion pump, although a mechanical pump alone may be satisfactory in many instances. If adequate sample is available (less than 1 cc. at standard conditions), the admission of gas may be completed by closing the pump connection and opening the sample bulb until the pressure, as measured on a mercury manometer behind the leak, is enough to give adequate ion intensity—commonly between 1 and 10 cm. of mercury. With a limited amount of sample (which may be as small as a few cubic millimeters at atmospheric pressure³⁵), the manifold must have as small a volume as possible, and it may be necessary to use a column of mercury in the form of a Toepler pump to compress the available gas behind the leak. The amount of gas actually used is very small, so that for any except minimum samples a large fraction may be recovered at the end of the analysis.

When mixtures of volatile substances which differ more widely in mass than do isotopes are to be analyzed, the sample system becomes more elaborate. This results from the requirement that the pressure behind the leak must be very much lower to insure molecular flow through the leak, and that this sample pressure must be accurately known for comparison with calibrating mixtures of the pure components. Since the sample pressure may be of the order of 0.04 mm. of mercury, it is usually more convenient and accurate to measure the pressure on a manometer connected with a small metering volume, and then to expand the sample into a much larger inlet reservoir connected to the leak. This is the system shown in figure 6. Special micromanometers for the direct measurement of pressures in the range from 10^{-3} to 10^{-1} mm. of mercury are also available.^{35a}

³⁵ H. G. Thode and R. L. Graham, *Can. J. Research*, **A25**, 1 (1947).

^{35a} W. S. Young and R. C. Taylor, *Ind. Eng. Chem., Anal. Ed.*, **19**, 133 (1947) I. E. Puddington, *Rev. Sci. Instruments*, **19**, 577 (1948).

When samples to be analyzed are liquids at ordinary pressure and temperature, special techniques must be used to introduce extremely small quantities to the inlet system. If the liquid has an appreciable vapor pressure, the sample will completely volatilize in the vacuum and the required pressure behind the leak may be adjusted by the weight of sample admitted. If the inlet volume is 1.0 liter, and a pressure of 0.04 mm. of mercury is required at 25° C., the quantity which must be admitted of a liquid having an average molecular weight of 100 is, from the gas laws, only about 0.26 mg. Taylor and Young³⁶ have shown that sintered glass disks covered with mercury are more convenient than stopcocks or break-seals in handling such small quantities. Such a disk is shown in the upper left of figure 6. Since the pores of the disk are too small to admit mercury, the valve is gastight to the vacuum in the direction shown. If a micropipette made of thermometer tubing is brought into contact with the first porous surface under the layer of mercury, liquid will transfer without loss to the atmosphere. Experience has shown that the engraving of the micropipette for the original thermometer calibration may be used as a measure of the sample admitted. For pure compounds, the pipette reading multiplied by the density of the liquid will be proportional to the weight of sample, and the response of the spectrometer may be expressed in terms of this weight instead of a pressure in the sample system. If suitable precautions are taken to avoid contaminating the mercury with sample and to prevent mercury from sticking to the pipette when it is withdrawn, a sintered glass valve is also a convenient closure for an auxiliary bottle in which calibrating mixtures of volatile liquids may be prepared by direct weighing. Gas mixtures below 1 atmosphere total pressure may also be held with this type of seal and admitted to the inlet system by direct contact between the sintered surfaces under mercury.

Since the amount of sample entering the spectrometer, and hence the intensity of ion beams observed, is a function of the pressure behind the leak, provision must be made to thermostat the inlet system if temperature variations are appreciable or if the sample contains relatively nonvolatile components which can be kept in the gas phase only at elevated temperatures. If any of the components condense out, the analyses will be meaningless.

For solid substances of low volatility, it is sometimes possible to obtain sufficient vapor for ionization by electron impact by charging the sample into a small furnace which becomes part of the ion source. This technique has been widely used for inorganic samples,^{37, 38} but requires opening the

³⁶ R. C. Taylor and W. S. Young, *Ind. Eng. Chem., Anal. Ed.*, **17**, 811 (1945).

³⁷ F. W. Aston, *Mass Spectra and Isotopes*. 2nd ed., Arnold, London, 1942, pp. 28, 70, *et seq.*

³⁸ A. O. Nier, *Phys. Rev.*, **53**, 282 (1938).

spectrometer tube and replacing the furnace after each sample has been examined. Deposits of material condensed in the ion source and the vacuum system will generally require frequent removal as well.

3. Gas Flow and Measurement of Pressure

For satisfactory operation of the mass spectrometer as an analytical instrument, certain conditions must be met regarding the flow of gas through the leak, the ionization chamber, and the vacuum pumps. For convenience, some of the conclusions derived from both theory and experience are listed below. They will be followed by a discussion of the principles on which some of them are based.

(1) Each component of the sample must exhibit a partial pressure in the ion source which is independent of other components present and proportional to its mole fraction in the sample.

(2) The pressure in the ionizing region of the source must usually be in the range from 10^{-3} to 10^{-5} mm. of mercury in order that sufficient ions may be formed by electron impact. This pressure must remain constant during the course of the analysis. The relatively high pressure necessary in the path of the electron beam where ions are actually formed may be produced, with a minimum pressure in the rest of the tube, if incoming gas is directed into this area rather than admitted to the ionization chamber and allowed to proceed in all directions by diffusion. When the gas is introduced as a well-defined molecular beam from an inlet tube as close as possible to the electron beam, and the ionization case is made as gastight as possible, a minimum amount of sample is required for adequate ion beam intensity and lower pressures are maintained elsewhere in the tube.

(3) The pressure in the analyzer must be as low as possible (10^{-6} mm. Hg or better) to minimize the scattering of ions by collisions with residual gas.

(4) Fast pumps, usually connected directly to the source region, are required to remove the un-ionized gas. If the effective speed of the pump is large compared to the amount of gas which it must handle, moderate variations in its pumping rate will have little influence on the pressure in the ionizing region. Washburn²⁹ states that it is possible to design the gas flow system so that a 100% increase in speed of the pump results in less than a 2% change in the pressure in the ion source.

(5) Mercury diffusion pumps preceded by cold traps are usually employed in preference to oil diffusion pumps, even though the latter may have greater pumping speeds. This choice reflects a fear of possible back-diffusion of gas fragments from the pumping oil which could not be dis-

²⁹ H. W. Washburn, "Mass Spectrometry," in *Physical Methods in Chemical and Metallurgical Analysis*. W. G. Berl, ed., Academic Press, New York, in press.

tinguished from the sample; ion peaks due to mercury are normally far separated from the sample spectrum. This choice is not absolute, however, and successful use of high-speed oil pumps has been reported by Brown, Mitchell, and Fowler,⁴⁰ and by others. The cold traps may require frequent cleaning to remove condensates. This is done with least interruption to operation if a valve between spectrometer and trap can be closed while the trap is warmed. Enclosed valves operated by an external magnet have been designed for this purpose.^{40a}

(6) Gas in the ionizing region must be kept from undergoing thermal decomposition on the hot filament followed by ionization in the usual way. By suitable pumping on the filament region and proper dimensioning of the ionization case, thermal decomposition products can be given less than a 1% probability of re-entering the ionization chamber.³⁹

(7) Materials for use in constructing ion sources and spectrometer tubes must be chosen to minimize surface adsorption, since this adsorption will lead not only to nonrepresentative partial pressures in the source, but also to cross-contamination of samples, or "memory" effects, and excessively long pump-out periods. External furnaces on the critical parts are required for periodic bake-out purposes.

(8) Although the intensity of the principal ion beams is one of the most sensitive measures of pressure in the tube, separate pressure-measuring equipment is convenient, if not actually essential. Ionization or Pirani gages attached to the source or pumping system are used to indicate when the pressure is low enough to turn on the main filament, and may be arranged through suitable relays to turn off all source voltages in case too much sample is admitted or a leak develops.⁴¹ A Philips ionization gage has been used in a number of cases instead of the more delicate triode type.²⁹ McLeod gages, usually connected between the pump and the cold trap, have also been used.

It has been pointed out by Washburn,⁴² by Washburn and Taylor,⁴³ and by Honig⁴⁴ that condition (1) is met when the dimensions of the leak and the pressure behind it are such that individual molecules pass through and into the ionization chamber without intermolecular collisions within the leak. This condition is

⁴⁰ H. Brown, J. J. Mitchell, and R. D. Fowler, *Rev. Sci. Instruments*, **12**, 435 (1941).

^{40a} H. W. Washburn, U. S. Pat. 2,431,355, to Consolidated Eng. Corp. (Nov. 25, 1947).

⁴¹ E. E. Hoskins, U. S. Pat. 2,374,205, to Consolidated Eng. Corp. (April 24, 1945).

⁴² H. W. Washburn, U. S. Pat. 2,412,236, to Consolidated Eng. Corp. (Dec. 10, 1946).

⁴³ H. W. Washburn and D. D. Taylor, U. S. Pat. 2,412,237, to Consolidated Eng. Corp. (Dec. 10, 1946).

⁴⁴ R. E. Honig, *J. Applied Phys.*, **16**, 646 (1945).

known as molecular flow and the laws governing it are those derived by Knudsen.⁴⁵ For a capillary tube of diameter D and length L , the volume of gas discharged per second at unit pressure is given by:⁴⁴

$$Q_m = 3800(D^3/L)(T/M)^{1/2}(p_1 - p_2) \text{ bar-cm.}^3/\text{sec.} \quad (12)$$

where T is the absolute temperature, M is the molecular weight on the scale $O^{16} = 16$, and p_1 and p_2 are, respectively, the pressures (in bars) on the upstream and downstream sides of the leak. For this equation to apply, it is found experimentally that the mean free path of gas molecules in the entire system, at an average pressure of $(p_1 + p_2)/2$ must be greater than 30 times the diameter of the opening, D . If carbon dioxide is the gas being considered, and the inlet reservoir is at a pressure (p_1) of 0.04 mm. of mercury with the ion source pressure (p_2) at 10^{-5} mm. of mercury, the mean free path at $(p_1 + p_2)/2$ is about 0.2 cm., and the leak diameter must be smaller than 0.007 cm. With this upper limit fixed, the actual diameter may be adjusted to give ample peak heights without interfering with the resolution or depleting the reservoir pressure too rapidly. If not enough gas is discharged by the maximum size opening, a number of leaks in parallel must be used in order to maintain the property of molecular flow.⁴⁶

From equation (12), it can be seen that although the rate of gas flow for each type of molecule is independent of other molecules present, it is inversely proportional to the square root of its own molecular weight. This results in a predictable fractionating effect at the leak for substances of varying molecular weight. For an extreme case, a mixture of equal molecular quantities of hydrogen and butane, the more rapid diffusion of hydrogen will result in the composition in the reservoir changing to 51.1 mole per cent butane : 48.9 mole per cent hydrogen after 1% of the butane has been pumped out.⁴⁴ When this "fractionating" type of leak is used, the effect must be minimized by using a large inlet reservoir close-coupled to the leak to favor mixing, and completing the analysis rapidly, so that p_i , during the course of the measurement, does not change by more than a fraction of 1%. Bleakney⁴⁷ has also pointed out that the same discrimination occurring at the leak will be expected at the diffusion pump, so that a constant relative concentration of each component should be attained in the ion source.

If pressures behind the leak are increased into the millimeter or centimeter mercury range, flow becomes viscous instead of molecular for a leak of any practical diameter, and the equation for the amount discharged contains the coefficient of viscosity but no longer contains the molecular weight. For a mixture of gases of widely different characteristics, the coefficient of viscous flow is found to change in an unpredictable manner with the abundance of the components. This means that the transport of a single component into the ion source (and so its partial pressure there) is dependent on other components, and fundamental requirement (1) is not obeyed.

When the gas to be analyzed contains only a single type of molecule in isotopic forms, the viscosity of the mixture does not change appreciably with composition

⁴⁵ M. Knudsen, *Ann. Physik*, **28**, 75, 999 (1909).

⁴⁶ H. W. Washburn, U. S. Pat. 2,387,786, to Consolidated Eng. Corp. (Oct. 30, 1945).

⁴⁷ W. Bleakney, *Phys. Rev.*, **41**, 32 (1932).

so that—for convenience—high pressures behind a so-called “nonfractionating” leak may be used.⁴⁴ In this case, however, viscous flow into the ion source followed by molecular flow (at low pressure) out of the source results in some slight discrimination favoring the heavier molecule, which has a slower rate of removal under the latter pumping condition. The particular design of nonfractionating leak used by Nier²⁹ is a copper capillary 20 inches long with an inside diameter of 0.006 inch. This is constricted by a pinchlamp at the end near the source. With a reservoir pressure high enough to give suitable ion beam intensities, the viscous flow of gas through the capillary is at a velocity greater than the rate of back-diffusion of fractionated products so that all molecules entering the capillary ultimately enter the spectrometer. This does not eliminate discrimination occurring at the reservoir end of the capillary or at the diffusion pump, but for isotope mixtures and for the continuous sampling of gas streams at high pressure where the fractionating leak cannot be used, the design has been found to be very suitable. A recording mass spectrometer for the continuous analysis of process gases in the uranium gaseous diffusion plant at Oak Ridge has been described⁴⁸ with an improved leak of this type.

Inghram¹⁶ has discussed the flow conditions in the mass spectrometer for various types of leaks and has pointed out the chief causes of discrimination.

4. Ion Sources

A. BASIS OF DESIGN

The functions of a mass spectrometer ion source have been outlined. For ionization by electron bombardment, factors in source design may be listed: (1) A beam of ionizing electrons must be provided, together with electrical circuits to measure its intensity and insure its constancy during analysis; adjustment of the energy of the ionizing electrons is also required. (2) The filament to supply electrons should be isolated from the ionizing region to minimize thermal cracking, and should be easily replaceable. (3) The region in which ionization by electron impact takes place must be carefully located with respect to the subsequent ion-accelerating system, and should lie in a space where the ion-accelerating potential is not changing rapidly. (4) Ionizing efficiency should be as high as possible so as to minimize the operating pressure and the amount of sample required. (5) The voltage for accelerating ions must be carefully stabilized, yet accurately and conveniently adjustable for scanning purposes. (6) The geometry of the ion source, including slit widths and alignment, and the location of the filament, should be capable of being checked before installation, and the entire unit should be accurately and firmly located with respect to the axis of the analyzer tube. (7) To obtain maximum ion intensities at a given sample pressure, the number of ion collimating plates should be kept to the mini-

⁴⁸ A. O. Nier *et al.*, *Anal. Chem.*, **20**, 188 (1948).

mum which will give the required resolution; slit widths should be as large as possible, subject to the same limitation. (8) Materials of construction must be nonmagnetic and must minimize gas adsorption and reactions producing nonconducting surface films. The use of gold-plated electrodes has been recommended.⁴⁹ (9) Since in gas analysis the spectra obtained are often functions of the temperature of the gas being analyzed, provision must be made to thermostat the ion source and to allow incoming gas to reach temperature equilibrium.

B. CONSTRUCTION OF ION SOURCE

An example of a very simple source suitable for isotope abundance measurements is shown in figure 7.⁵⁰ Other references cited in this section should be consulted for details of alternate designs. Ion sources used in commercial instruments for gas analysis have not been described in detail,^{50a} but they do not appear to differ fundamentally from that shown schematically in figure 6, although many refinements have been introduced, with claimed improvement in operating characteristics. Such sources are generally serviced by the manufacturer on a replacement basis, so that the user does not need to adjust or repair them.

The ion source shown in figure 7 consists of an assembly of plates of non-magnetic Nichrome V approximately 3.5 cm. in diameter, separated by glass spacing washers and held together by small Nichrome V bolts. To supply electrons for ion production, a hot tungsten filament in the shape of a hairpin is placed at *F*, behind a shield *S*₁. The filament is heated, most simply by two 6-volt storage batteries with rheostat control, but more conveniently by a power-supply unit which automatically regulates the filament heating current to maintain a constant rate of electron emission, which can be adjusted to any desired value. A potential of from 50 to 150 v. from radio "B" batteries or from a tap in the emission regulator, if one is used, is applied between the filament and *S*₁, making the latter positive. Some of the electrons emitted by the filament pass through *S*₁, through the ionizing region between plates *B* and *C*, and finally reach trap plate *T*. The electron current to *T* may amount to 20 microamp. or more and must be kept constant during the course of an analysis. Suitable electronic circuits for electron acceleration and emission regulation are described by Nier,²⁹ by Graham, Harkness, and Thode,⁵¹ and by Hipple, Grove, and Hickam.³³

⁴⁹ Westinghouse Elec. & Mfg. Co., *Mass Spectrometer Descriptive Data*, 85-940, p. 5.

⁵⁰ A. O. Nier, *Rev. Sci. Instruments*, **11**, 212 (1940).

^{50a} See H. W. Washburn, U. S. Pats. 2,378,962, 2,413,668, 2,444,968, 2,450,462, 2,472,870, 2,475,653, to Consolidated Eng. Corp.

⁵¹ R. L. Graham, A. L. Harkness, and H. G. Thode, *J. Sci. Instruments*, **24**, 119 (1947).

Ions formed in a narrow region between plates *B* and *C* (Fig. 7A) are accelerated toward *C* by a potential of a few volts. Certain ions pass through *S*₁ and are then strongly accelerated toward plate *D* by from 800 to 1000 v. This voltage may be supplied from a bank of "B" batteries (the very low current drain permits battery life up to the normal "shelf life") or, more flexibly, by a stabilized transformer-rectifier unit.^{29, 51} The

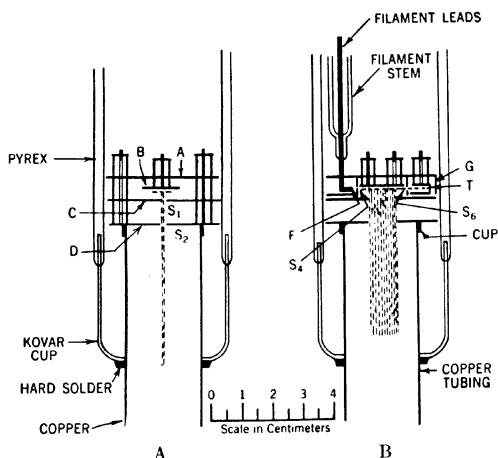


Fig. 7.—Two cross-sectional views of Nier's ion source.⁵⁰ In A, the filament stem and electron trap box are not shown. In B, the studs holding plates *A*, *C*, and *D* are omitted for the sake of clarity.

exact voltage applied from a voltage divider determines what mass ion will be brought to focus at the collector slit. This circuit is shown in figure 6, connected to corresponding electrodes of the ion source. For automatic voltage scanning, the high voltage may be allowed to decay at a suitable rate by a capacitor-resistor network,^{52, 53} or it may be varied by a motor-driven rheostat.⁵¹ The m/e value of any peak observed may be determined by the use of equation (9), page 1996, or may be indicated by a "mass marker."⁵⁴

The entire ion source is mounted on the end of the analyzer tube, where it is held in exact alignment by locating pins and bolts, or by a carefully

⁵² C. E. Berry, *Electronic Inds.*, **3**, No. 6, 94 (1944).

⁵³ R. C. Olesen, U. S. Pat. 2,397,560, to Consolidated Eng. Corp. (April 2, 1946).

⁵⁴ R. V. Langmuir, U. S. Pats. 2,376,877 and 2,378,936, to Consolidated Eng. Corp. C. E. Berry, U. S. Pat. 2,370,631, to Consolidated Eng. Corp.

fitted cup arrangement, as shown in figure 7. A vacuum-tight envelope, commonly of glass, surrounds the complete source, with electrical leads brought in as shown in figure 6. When the source is to be more easily removable, it may be mounted from the lid of the enclosing envelope, which is made of metal and sealed with a Teflon gasket in Nier's latest design.²⁹

C. AUXILIARY MAGNETS

In order to locate the ionizing region at a uniform distance from plate *C* (and so have all ions start at a uniform potential and follow equivalent trajectories), the electron beam is usually aligned by a magnet external to the source having its field parallel to the electron beam. This field of about 150 gauss, in conjunction with slit *S*₄ (Fig. 7B), collimates the electron beam, and greatly increases the number of electrons which pass into the ionizing region. Coggeshall and Jordan⁸ have described a source not requiring such a magnet, and in the 180° instrument the main magnetic field serves this purpose. Auxiliary magnets are usually of the permanent type, and their location is usually critical.

D. METASTABLE IONS

In the usual spectrometer, positively charged ions are accelerated from a positive potential to zero. This permits the analyzer tube and collector to operate at ground potential and ions reach the collector with the full energy acquired in the source. In such a source, if ions of mass *m*₀ are formed in a metastable condition so that they spontaneously decompose (after being accelerated but before they enter the analyzer) into a second ion of mass *m* and neutral fragments of mass (*m*₀ − *m*), it has been shown that the new ion is observed at an apparent mass, *m*^{*}, given by:

$$m^* = m^2/m_0 \quad (13)$$

The C₃H₇⁺ ion formed by the spontaneous reaction, C₄H₁₀⁺ (58) → C₃H₇⁺ (43) + CH₃ (15), thus gives a peak at apparent mass 31.9; similarly, other metastable ions⁵⁵ may contribute to broadening other peaks or to creating a diffuse background.

In a series of papers, Hipple *et al.*⁵⁶⁻⁵⁸ have shown that the effect of metastable ions can be eliminated by accelerating from +1 or +2 v. above ground at the ion source to a large negative potential at the analyzer. The collector is again at ground potential so that all "normal" ions are decelerated as they approach the collector, and reach it with an energy of only a few electron volts. In a metastable decomposition, the original kinetic energy is divided between the new ion and the

⁵⁵ V. H. Dibeler, C. E. Wise, and F. L. Mohler, *Phys. Rev.*, **71**, 381 (1947). Also E. G. Bloom, F. L. Mohler, J. H. Lengel, and C. E. Wise, *J. Research Natl. Bur. Standards*, **40**, 437 (1948).

⁵⁶ J. A. Hipple and E. U. Condon, *Phys. Rev.*, **68**, 54 (1945).

⁵⁷ J. A. Hipple, R. E. Fox, and E. U. Condon, *Phys. Rev.*, **69**, 347 (1946).

⁵⁸ J. A. Hipple, *Phys. Rev.*, **71**, 594 (1947); *J. Phys. & Colloid Chem.*, **52**, 456 (1948).

neutral fragments so that the former carries only a fraction of the energy gained in acceleration. Since the positive ion which has lost energy is decelerated to a complete stop and then repelled by the grounded collector, the effect of fractional mass peaks is eliminated. In examining many organic gases, it is claimed that the sharpness of the spectra obtained can be considerably improved by this method. When minor constituents of a mixture are to be determined, the pressure may be increased by as much as a factor of ten without scattered ions resulting in a loss of resolution at the collector. In most existing instruments, however, the power supplies are either not designed for operation with the positive side grounded, or the analyzer tube cannot be conveniently operated at a high negative potential, so that the change is not as simple to make as it might appear. A description of the experimental arrangement found most suitable for collecting the decelerated ions, and for preventing the unwanted collection of secondary electrons which arise from collisions with the retarding plates, has been given by Fox and Hipple.⁵⁹

E. DISCRIMINATION BY ION SOURCE

If absolute abundance measurements are to be carried out, it is necessary to eliminate or correct for any discrimination between ions which occurs in the source. The causes of such discrimination are not completely understood, though Inghram,¹⁶ Jordan and Coggeshall,⁶⁰ and Coggeshall⁶¹ discussed a number of factors inherent in the design, and Washburn and Berry⁶² and Washburn^{30,39} discussed the effect of initial kinetic energies on the number of ions drawn through the accelerating system. When ions are formed with initial kinetic energy, their motion, in general, will not be perpendicular to the collimating plates, and few ions of high initial energy will contribute to the useful ion beam. In an ion source patented by West,⁶³ the collimating slits are intentionally offset so that ions formed with initial kinetic energy are allowed to pass through, while those accelerated without initial energy are not. It is claimed that this source will permit the measurement of ion abundances as a function of initial energies. The same problem can also be studied by means of retarding potentials, as described above.

There is little that the user of a mass spectrometer can do to control these discriminatory effects beyond adjusting focusing conditions to the optimum and being sure of proper mechanical alignment. Their importance is minimized in most applications if an observed ratio of abundances is compared with a standard ratio determined under similar conditions.⁶⁴ In

⁵⁹ R. E. Fox and J. A. Hipple, *Rev. Sci. Instruments*, **19**, 462 (1948).

⁶⁰ E. B. Jordan and N. D. Coggeshall, *J. Applied Phys.*, **13**, 539 (1942).

⁶¹ N. D. Coggeshall, *J. Chem. Phys.*, **12**, 19 (1944).

⁶² H. W. Washburn and C. E. Berry, *Phys. Rev.*, **70**, 559 (1946).

⁶³ S. S. West, U. S. Pat. 2,427,484, to Stanolind Oil & Gas Co. (Sept. 16, 1947).

⁶⁴ A. O. Nier and E. A. Gulbransen, *J. Am. Chem. Soc.*, **61**, 697 (1939).

isotope work, this is done by referring all ratios to a standard sample which is measured frequently. In gas analysis, frequent calibrating runs with pure components provide a similar comparison. In these cases, *changes* in the ratio are of significance and discrimination effects are of less importance.

When mass scanning is accomplished by varying the accelerating voltage (as opposed to varying the magnetic field), one fairly sizable discrimination effect is usually noted, although neither its magnitude nor its sign can always be predicted. From the schematic ion-accelerating circuit shown in figure 6, it may be seen that, as the total accelerating voltage is changed, the voltage between the uppermost plate (the so-called ion pusher) and the first ion slit also changes. As a result, an ion of lighter mass (m_1), accelerated at a higher voltage, receives a larger initial "push" than the next heavier ion (m_2), when the scanning has reduced the accelerating voltage so as to bring it to the collector. This difference in initial acceleration usually results in more ions of mass m_1 appearing at the collector than of mass m_2 when the latter is focused, even though the two species are equally abundant in the electron beam. Normally, this discrimination favors the lighter ion to an extent of 1% or more when ions such as $\text{C}^{12}\text{O}_2^+$ and $\text{C}^{13}\text{O}_2^+$ are compared. The effect is usually larger in sector instruments than in the 180° type. Inghram⁶⁵ has found this effect to be as great as 20% when comparing isotopes differing in mass by 10%. This was especially true under conditions of poor focus.

This effect was observed by Dempster⁶⁶ in 1922, using a source in which the "pusher" electrode was omitted and ions presumably reached the first slit entirely by a process of diffusion. Here, the "voltage effect" observed was ascribed to the penetration of the varying ion-accelerating field through the slit in the first plate. Dempster's solution was to place a grid of fine wires across the slit to reduce the stray field. That the effect is not entirely electrostatic is suggested by the observation of negative voltage effects, in which more ions, rather than fewer, appear to be accelerated at lower voltages. The magnitude of the effect has also been found to vary between individual sources of supposedly identical design, and from time to time in the same source.

A general method for elimination of the voltage effect is to hold all source conditions constant, including the accelerating voltage, and to scan by varying the magnetic field. This is satisfactory for any application where a continuous scan at a steady rate may be used, as with an automatic recorder, but is less adaptable to manual operation where the time constant

⁶⁵ M. G. Inghram, *Phys. Rev.*, **70**, 653 (1946).

⁶⁶ A. J. Dempster, *Phys. Rev.*, **20**, 631 (1922).

of the magnetic circuit makes it difficult to set the field accurately to a desired value and to return to this value later.

5. Magnetic Analyzers

Figure 8 is a cross-sectional view of a Nier 60° spectrometer⁵⁰ showing the relation of the source and collector to the sector-shaped magnetic field and to the energizing coils of the electromagnet. For purposes of baking out the analyzer tube, the magnet is mounted on a dolly which can be moved out of position, although this is seldom necessary if heat is applied to the ends of the analyzer. The tube itself is usually of copper with glass-to-metal

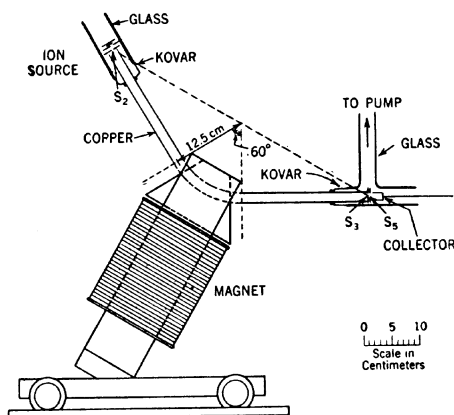


Fig. 8.—Cross-sectional view of the entire Nier 60° mass spectrometer.

seals to the envelopes at each end. The copper may be flattened slightly in the bent portion, to reduce the gap required in the magnet, as shown in figure 5.

In the 180° instrument, the outer envelope may be of glass over the entire length, but in this case a rigid metallic liner of copper must be slipped in from one end of the arc to provide electrostatic shielding for the ion beam and to provide a base for alignment of source and receiver.⁴ Semi-flexible stove-pipe shields inside the glass have also been used,¹⁰ but in this case the glass itself must provide the necessary alignment.

In order to reduce the magnet gap still further, a number of designers of sector instruments have enclosed and evacuated the space between the actual pole pieces, thus eliminating a separate envelope in the analyzer section. Vacuum seals are provided at each side of the magnet for at-

tachment of straight tubes which carry the source and the receiver.^{8, 9, 67}

The strength of magnetic field commonly used varies from 2000 to 6000 gauss, with provision made to reduce this in case very light ions, *e.g.*, hydrogen and helium, are to be analyzed. For small fields of this strength, permanent magnets of the Alnico type may be used^{30, 68} which are very suitable from the standpoint of constancy, but which are not easily adapted to magnetic scanning. In order to reduce the Alnico field for the analysis of hydrogen, Washburn³⁰ has described a magnetic shunt.

Electromagnets may be powered from storage batteries,⁵⁰ electronic supplies,^{29, 51, 52} or from electronically regulated motor generator sets,⁶⁹ depending on their size. The magnet current must be highly stable. It has been shown⁵¹ that resolution of mass 200 from mass 201 requires the magnetic field constant to 1 part in 20,000 for the period required for the analysis. In general, magnet excitation requires from 10 to 200 or more watts, and all except the largest magnets may be powered by vacuum-tube regulated supplies. For the *Consolidated* 180° instrument, one supply utilized twenty-two Type 6A3 tubes in parallel to pass regulated currents up to 1.5 amp.⁵² A design of 180° magnet which produces a uniform field only in a ring at the location of the semicircular analyzer tube, with a resultant saving in size and power required, has been described.⁷⁰ For automatic scanning, a motor-driven spiral potentiometer may provide a variable signal to the regulating circuit,⁵¹ or an electronic network in parallel with the magnet may be varied to divide the constant total output of the regulator.³³ For greatest convenience in identifying the m/e values of ion peaks observed while scanning magnetically, a fluxmeter³³ is required since magnet current and field strength do not bear an exact instantaneous relation to each other because of hysteresis. The output of the fluxmeter may trip preset relays which indicate or record the passing of selected m/e values in the scanning.

6. Ion-Current Measurements

A. COLLECTOR SYSTEMS

The simplest and most generally used ion collector is shown in figure 8. The exit slit, S_s , defines the actual point of focus and is usually made slightly wider than the image of the source slit which appears at this point. With such a slit, the shape of the ion peak, when plotted against m/e , or the accelerating voltage, has a flattened top corresponding to the interval during which the whole peak is entering the exit slit. This flat top facilitates measurement of the peak height by any of the methods to be described.

⁶⁷ R. E. Fox, J. A. Hipple, and T. W. Williams, *Phys. Rev.*, **65**, 353 (1944).

⁶⁸ J. E. Taylor, *Rev. Sci. Instruments*, **15**, 1 (1944).

⁶⁹ W. M. Schwarz, *Rev. Sci. Instruments*, **13**, 213 (1942).

⁷⁰ H. S. Anker, *Rev. Sci. Instruments*, **19**, 440 (1948).

If the slit is too wide, the resolution will be limited to lower masses, as indicated in equation (11) on page 1999. If the exit slit is narrower than the focused beam, not all of the ions of a given m/e will reach the collector when the beam is centered on the slit. Under proper conditions of focusing, however, a measurement of the ion currents corresponding to maximum peak heights is found to be proportional to the total beam intensity. An exit slit of adjustable width may be operated through a metal bellows.⁵⁹

Behind the exit slit, a second, wider slit, S_2 , is usually operated at a potential of -22.5 or -45 volts with respect to the exit slit, to prevent secondary electrons caused by ion bombardment from reaching the collector. The collector electrode itself is usually supported only by its own lead-in wire to minimize electrical leakage to the other electrodes. When ions are decelerated before collection, more elaborate shielding and electron traps are required.⁵⁹

On the outside of the envelope the collector lead is carried as directly as possible to the grid of the first amplifier tube. This tube is often contained in an evacuated housing located adjacent to the collector so that the lead carrying the minute ion current may be as short and as well shielded as possible and so that moisture may be kept away from the tube and the high input grid resistance. The input grid resistor usually has a resistance of 10^9 to 10^{10} ohms. Polarization effects and non-ohmic response to varying ion currents⁶⁵ are often encountered with such high-megohm components, and selection of individual resistors based on their performance in the circuit may be necessary.

B. AMPLIFIERS

Sensitive d.-c. amplifiers used with the mass spectrometer may be of the electrometer-tube type,⁷¹ an FP-54 or similar tube being utilized in a single stage of amplification, with the output operating a sensitive galvanometer. This arrangement is discussed and a typical circuit given by West in Chapter XXI of this volume, and similar amplifiers used with ionization chambers are described by Bale and Bonner in Chapter XXX. For small deflections, the galvanometer scale reading is proportional to the ion-current intensity, but the linearity of the amplifier falls off with increasing ion beams so that a potentiometer of the "Student" type is often used to apply a counter-e. m. f. to the input of the amplifier so as to make it a null instrument. When adjusted for zero deflection of the galvanometer, the potentiometer setting is proportional to the ion-beam intensity, and hence to the abundance of the particular ion being observed.

Alternate types of amplifiers have been described, including several that

⁷¹ D. B. Penick, *Rev. Sci. Instruments*, **6**, 115 (1935).

operate from a.-c. lines. The requirements are severe since ion currents may vary down to 10^{-14} amp., thus requiring exceptional zero stability and a long range of response. The most satisfactory type, used in several recent instruments, is an inverse feedback circuit using an electrometer tube preamplifier with several additional stages of amplification.^{29, 51} This amplifier has a linear response over a wide range so that the output may be read from a suitable meter or from the chart of a recorder.

An adaptation of the electron-multiplier tube has been suggested for the measurement of ion beams of very low intensity, but this appears to have many practical difficulties.¹⁶ More important is the use of vibrating-reed or dynamic-condenser electrometers⁷² which convert the ion current to an alternating signal which can then be amplified by the more stable types of a.-c. amplifiers. Similar circuits are described in chapter XXX for measuring small ionization currents in the measurement of radioactivity. Instead of converting d. c. to a. c. in the amplifier, the ion beam itself may be pulsed by alternating voltages applied in the ion source, and the intermittent signal at the collector amplified with a.-c. circuits having suitable stability and time constants.^{72a}

For the measurement of isotope ratios, the use of a multiple-collector system which will collect simultaneously the ion beams of interest has been widely used.⁷³ Fed into parallel amplifiers, two ion currents may be compared directly by balancing a measured fraction of the larger output against the smaller to obtain a null reading on a galvanometer.^{30, 74} The fraction of the larger required to give this balance is then proportional to the true ratio, the proportionality constant depending on the relative sensitivity of the two amplifiers and on the fraction of the total beam received by each of the collectors. Measurement of the same ratio on standard samples provides a method of calibration. The method is of particular value when small changes in an isotope ratio near the normal value are to be measured, since the simultaneous measurement of the two peaks cancels out any small changes in ionization efficiency. When samples differing widely from normal are encountered, it is necessary to calibrate by reference to standard samples whose isotope content is accurately known. This calibration is found to change with time, so that for the most precise work, standard samples must be run alternately with unknowns or, as a minimum, several times a day. The dual-collector system can, or course, be run using only one collector and one amplifier when this is more convenient.

⁷² H. Palevsky, R. K. Swank, and R. Grenchik, *Rev. Sci. Instruments*, **18**, 298 (1947).

^{72a} R. V. Langmuir, U. S. Pats. 2,370,673, 2,457,162, 2,463,544, and 2,463,545, to Consolidated Eng. Corp.

⁷³ H. A. Strauss, *Phys. Rev.*, **59**, 430 (1941). H. Hoover, Jr., U. S. Pat. 2,341,551, to Consolidated Eng. Corp. (Feb. 15, 1944).

⁷⁴ A. O. Nier, E. P. Ney, and M. G. Inghram, *Rev. Sci. Instruments*, **18**, 294 (1947). See also U. S. Pat. 2,456,426 (Dec. 14, 1948).

C. RECORDERS

From the preceding description, it would appear feasible to record any mass spectrum by a repetition of the following manual processes: (1) adjusting the scanning control to bring a peak to focus at the collector, (2) applying the counter-e. m. f. necessary to balance out the peak, (3) recording the potentiometer reading as a measure of the ion abundance, and (4) checking the zero of the amplifier-galvanometer system, with no ions being received. In practice, it is found that automatic scanning and automatic recording of ion peak heights are highly desirable if more than a

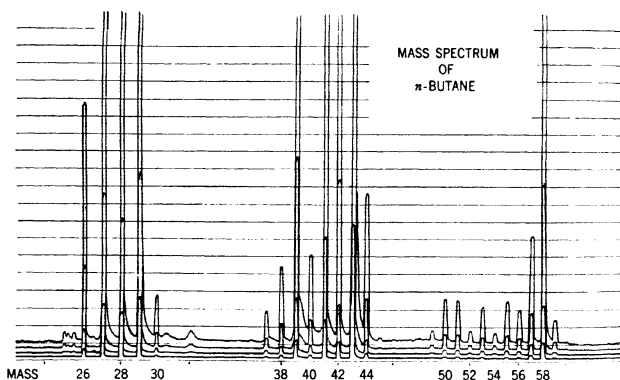


Fig. 9.—Mass spectrum of *n*-butane traced photographically by four-element galvanometer (courtesy Consolidated Engineering Corp.).

few ionic masses are of interest. Because of inevitable drifts in ion intensity with time, especially if small samples are being analyzed, it is desirable to complete an analysis as quickly as possible. While manual operation can be made very rapid when successive comparisons of the same two peaks are involved, the automatic method is quicker and involves much less chance for personal error when examining a complex spectrum.

At least four general types of recorders have been used as output meters on ion-current amplifiers. Smith, Lozier, Smith, and Bleakney⁷⁵ used photographic recording of light reflected from a galvanometer mirror onto a strip of sensitized paper. Washburn, Wiley, and Rock^{76, 77} have described

⁷⁵ P. T. Smith, W. W. Lozier, L. G. Smith, and W. Bleakney, *Rev. Sci. Instruments*, **8**, 51 (1937).

⁷⁶ H. W. Washburn, H. F. Wiley, and S. M. Rock, *Ind. Eng. Chem., Anal. Ed.*, **15**, 541 (1943).

⁷⁷ E. E. Hoskins and R. V. Langmuir, U. S. Pat. 2,380,439, to Consolidated Eng. Corp. (July 31, 1945).

a four-element galvanometer in which four beams of light are deflected simultaneously by separate galvanometers having different sensitivities. A typical recording produced by this instrument is shown in figure 9. The upper trace is produced by a galvanometer of unit sensitivity with other traces showing one third, one tenth, and one thirtieth of this deflection. Peaks are read on the most sensitive trace which remains on scale and are multiplied by the appropriate sensitivity factor. The lines of the scale are recorded photographically at the same time as the peaks, so that distortions of the paper during processing and drying need not be considered. This recorder is very fast and can record two octaves of peaks, as from mass 15 to mass 60, or from mass 25 to mass 100, in three minutes.

A photopen recorder⁷⁸ using a double cathode phototube mounted on a pen carriage has been described by Müller in Chapter XXVIII. A pen speed of 12.7 cm. per sec. across a 25-cm. chart, with a minimum response of 0.1 mm., was obtained in recording mass spectra.

Fast mechanical recorders (of the *Speedomax G* type, manufactured by Leeds and Northrup) have also been adapted to mass spectrometer recording.^{33, 79} In one application, a logarithmic response is used to compress the scale and a series of relays and a shunt selector then act to re-scan the peak on an appropriate linear scale. In the recorder described by Lossing, Shields, and Thode the input signal to the recorder acts to trip thyratrons whenever the pen approaches full scale, and these in turn operate relays which change the sensitivity of the recorder. An auxiliary receiver electrode, with its own pilot amplifier, may also be used to adjust the recorder sensitivity before each ion beam reaches the main collector.^{79a} With such instruments, up to about six mass units may be scanned per minute at a uniform rate, and this may be increased if a more rapid rate of scanning is automatically used between peaks.

When only certain peaks in the spectrum are of interest and a continuous record is desired of how these vary, a multipoint recorder may be used, with a motor-driven switch which will automatically select the proper accelerating voltage to bring each mass to the collector and to record its abundance. Such a system has been used to record the composition of a continuously variable stream of process gas.⁴⁸

The ultimate in speed of scanning is obtained when the pattern of ion peaks for a given region of the spectrum is shown on a cathode-ray oscilloscope.⁸⁰ Forrester and Whalley⁸¹ have shown that for highest sensitivity,

⁷⁸ D. J. Pompeo and C. J. Penther, *Rev. Sci. Instruments*, **13**, 218 (1942).

⁷⁹ F. P. Lossing, R. B. Shields, and H. G. Thode, *Can. J. Research*, **B25**, 397 (1947).

^{79a} C. H. Schlesman, U. S. Pat. 2,470,745, to Socony Vacuum Oil Co. (May 17, 1949).

B. W. Thomas, U. S. Pat. 2,476,005, to Standard Oil Dev. Co. (July 12, 1949).

⁸⁰ J. A. Hipple, U. S. Pat. 2,331,189, to Westinghouse Elec. & Mfg. Corp. (Oct. 5, 1943).

⁸¹ A. T. Forrester and W. B. Whalley, *Rev. Sci. Instruments*, **17**, 549 (1946).

the scanning voltage (which they vary in a saw-tooth manner by motor-driven rheostats and which is synchronized with the horizontal sweep of the oscilloscope) should change relatively slowly. Best results for very small ion currents were given by sweeps requiring from 1 to 30 seconds. An oscilloscope with a long-persistence screen was used to show mass patterns covering 200 mass units, or any part of the spectrum could be examined on a larger scale. Siri⁸² has described a compact a.-c. operated, 180° spectrometer in which a linear sweep voltage of from 0 to 200 v. at 200 cycles per second is imposed on an adjustable, d.-c. accelerating voltage. This allows a choice of both the region to be scanned and of the width of scan. The output of special amplifying circuits is applied to the oscilloscope. Resolution is adequate up to mass 65, and a sensitivity of 5×10^{-12} amp. per inch of deflection is obtained. In measuring peak heights (by the use of a voltage divider to reduce larger peaks to a standard height), an accuracy of from 2 to 5% is claimed. While not nearly as accurate as other recorders for small ion currents, instruments of this type may have important applications in studying transient effects where the appearance and disappearance of a component of interest may take place very rapidly. Respiratory gases have already been examined in this way for rapidly disappearing components.

IV. APPLICATIONS

1. Tracer Technique with Stable Isotopes

A. PRINCIPLES—AVAILABILITY OF TRACERS

The preceding chapter, on determination of radioactivity, has discussed fundamentals of the tracer technique. The extension of this method to the use of separated stable isotopes has received a great deal of attention, particularly in biochemistry, and only a brief review of its technique can be given here.⁸³

⁸² W. Siri, *Rev. Sci. Instruments*, **18**, 540 (1947).

⁸³ See the following for recent summaries of general interest: D. Rittenberg and D. Shemin (pp. 261-76), in *Currents in Biochemical Research*, D. E. Green, ed., Interscience, New York, 1946. D. W. Wilson, A. O. Nier, and S. P. Reimann, eds., *Preparation and Measurement of Isotopic Tracers*, Edwards, Ann Arbor, 1946. A. S. Keston (pp. 1-18) and H. S. Taylor (pp. 19-42) in *Advances in Nuclear Chemistry and Theoretical Organic Chemistry*, R. Burk and O. Grummitt, eds., Interscience, New York, 1945. J. M. Buchanan and A. B. Hastings, *Physiol. Revs.*, **26**, 120-55 (1946). H. G. Wood, *ibid.*, **26**, 198-246 (1946). J. R. Loofbouroow, *Revs. Modern Phys.*, **12**, 280 (1940). J. Sacks, *Chem. Revs.*, **42**, 411 (1948). G. Popjak, *Science Progress*, **36**, 239 (1948). *Symposium on the Use of Isotopes in Biology and Medicine*, Univ. Wisconsin Press, Madison, 1948. N. S. Radin, *Nucleonics*, **1**, No. 1, 24-33; No. 2, 48-59 (1947). M. D. Kamen, *Radioactive Tracers in Biology*, Academic Press, New York, 1947; also *Ann. Rev. Biochem.*

Of the total number of elements, only a few consist of a single isotope; beryllium, fluorine, sodium, and aluminum are examples of this class. A larger number of elements consist of two stable isotopes, while the remainder have three or more isotopes. To a remarkable extent the abundance ratio of these isotopes is a constant which does not depend upon the source of the element or its previous history, although variations in the C^{13} , S^{34} , and B^{10} content of natural materials have been measured.⁸⁴ Animal organisms do not discriminate appreciably between the mass species, except in the case of deuterium where the 100% increase in mass may affect the behavior; this is even more probable when radioactive H^3 is used.

When elements such as carbon and nitrogen are labeled, it is usually assumed that any fractionation of the tracer isotope in the reaction under study will be slight in comparison with other sources of error. Recent work suggests that this assumption, although generally true, should be critically examined for each reaction system of interest. For example, in the thermal cracking of propane containing C^{13} in the 1-position, it was found that the over-all reaction indicated an 8% greater frequency of breaking a $C^{12}-C^{12}$ bond than a $C^{12}-C^{13}$ bond.⁸⁵ Such a difference as this must be considered in quantitative tracer experiments.

If an element with an abnormal isotope ratio is synthesized into a compound and this is used in a chemical reaction or fed to an experimental animal, the presence of an abnormal ratio in a product of the chemical reaction, or in a metabolic product, is evidence of the course taken by the element in question. The mass spectrometer, with suitable methods of preparing samples, can be used to analyze isotope ratios in any tracer element, and the results obtained by its use are unusually free from possible misinterpretations.

To be used as a tracer, an element must be artificially enriched in one of its isotopes. It is fortunate that methods are available by which this may be done, although the process is always a slow and difficult one. The lighter elements, particularly hydrogen, carbon, nitrogen, oxygen, and sulfur, are the ones of interest to the organic chemist; methods are well known for the preparation of "heavy" samples of these for use as tracers.^{86, 87} Separation of the stable isotopes of the heavier elements is more difficult, as has been shown in the wartime work with uranium, and radioactive tracers are more often used. Table I shows the isotopic composition of elements of interest. Radioactive isotopes are shown as well as stable ones, along with commercial sources in the United States in 1949.

16, 631 (1947). G. Hevesy, *Radioactive Indicators*, Interscience, New York, 1948. E. C. Dougherty and J. H. Lawrence (Chapter I) and B. Vennesland (Chapter II), in *Advances in Biological and Medical Physics*, Vol. I, J. H. Lawrence and J. G. Hamilton, eds., Academic Press, New York, 1948.

⁸⁴ B. F. Murphey and A. O. Nier, *Phys. Rev.*, **59**, 771 (1941). H. G. Thode *et al.*, *Can. J. Research*, **B27**, 361 (1949); also *J. Am. Chem. Soc.*, **70**, 3008 (1948).

⁸⁵ D. P. Stevenson, C. D. Wagner, O. Beeck, and J. W. Otvos, *J. Chem. Phys.*, **16**, 993 (1948).

⁸⁶ H. C. Urey, *Reports on Progress in Physics*, **6**, 48 (1939); *J. Applied Phys.*, **12**, 270 (1941); *Elec. Eng.*, **67**, 581 (1948).

⁸⁷ D. W. Stewart, *Nucleonics*, **1**, No. 2, 18 (1947). G. W. Dunlap and R. M. Lichtenstein, *Elec. Eng.*, **67**, 469 (1948). See also *U. S. Naval Med. Bull. Supplement*, Mar.-April, 1948, pp. 6-16, 28-41, 205-209.

TABLE I
STABLE AND RADIOACTIVE ISOTOPES

Element	Mass	Abundance (if stable)	Half-life (if radioactive)
Hydrogen	1	99.98	—
	2 ^a	0.02	—
	3 ^b	—	12 years
Carbon	10	—	8.8 sec.
	11	—	20.5 min.
	12	98.9	—
	13 ^c	1.1	—
	14 ^b	—	5100 years
Nitrogen	13	—	9.93 min.
	14	99.62	—
	15 ^c	0.38	—
	16	—	8.0 sec.
	15	—	126 sec.
Oxygen	16	99.76	—
	17	0.04	—
	18 ^d	0.20	—
	19	—	31 sec.
	31	—	3.2 sec.
Sulfur	32	95.1	—
	33	0.74	—
	34	4.2	—
	35 ^b	—	87.1 days
	36	0.016	—

^a Distributed by *Stuart Oxygen Co.*, San Francisco, Calif., on allocation by Isotopes Branch, U. S. Atomic Energy Commission, Oak Ridge, Tenn.

^b Isotopes Branch, U. S. Atomic Energy Commission, Oak Ridge, Tenn.

^c Research Laboratories, *Eastman Kodak Co.*, Rochester, N. Y.

^d Small amounts at low concentration available on same basis as H² (see *a* above).

An examination of the table reveals that radioactive isotopes of nitrogen and oxygen are too short-lived for extensive experimentation, so that stable N¹⁵ and O¹⁸ must be used. In the case of hydrogen, carbon, and sulfur, a choice exists between radioactive and stable forms, although separated S³⁴ or S³⁶ are not available commercially at present.

B. GENERAL APPLICATIONS

Some of the general applications of tracers which are useful in organic chemistry as well as in physiology and biochemistry are: (1) detecting the conversion of one compound to another, (2) studying the mechanism of reactions by identifying the formation and conversion of intermediates, (3) measuring the rates of reactions, especially under equilibrium conditions

where there is no net change in the amount of product, and (4) using the isotope dilution technique to determine the amount of a component in a mixture.

In many experiments, it may be desirable to use two different tracers, either one radioactive and one stable isotope of a single element or labeled forms of two different elements, in a single experiment. Most simply, such "double labeling" will allow two structurally different parts of a single molecule to be followed simultaneously through a complex reaction, with a resulting saving of time over two successive experiments, each using a single label. More important, Branson⁸⁸ has pointed out that in determining the rate of biochemical reactions leading to the "turnover" of material present in the body in constant amount, the rates of formation from a suspected precursor must be shown to be equal to the rate of disappearance of the compound. While this can be done with successive experiments using labeled precursor and labeled compound, the use of different biological systems, with possible differences in kinetic characteristics, makes the results somewhat uncertain. If double labeling is employed, both sets of information may be obtained at the same time in a single biological system.

C. ANALYSIS BY ISOTOPE DILUTION

This method of quantitative analysis has been applied principally to the analysis of complex mixtures of amino and fatty acids. It can, however, be extended to any other system in which small fractions of each component can be isolated in a pure state, but in which quantitative separations are not easily made. The method, as developed by Rittenberg and Foster,⁸⁹ requires that synthetic samples of each substance to be determined be prepared with one element in a "heavy" state. Thus, in amino-acid analysis, samples of the various acids would be prepared containing nitrogen enriched in N^{15} , or carbon enriched in C^{13} . When these synthetic acids are added to the unknown mixture they become indistinguishable from the original components. If a fractionation is now carried out to obtain, in very small yield if necessary, a pure sample of each component, the isotope ratio can again be determined on this recovered material. If the abnormal ratio (when compared with the sample added) is now found to be more nearly normal, the dilution will be a measure of the amount of the particular acid originally present. In organic systems, for which quan-

⁸⁸ H. Branson, *Science*, **106**, 404 (1947).

⁸⁹ R. Schoenheimer, S. Ratner, and D. Rittenberg, *J. Biol. Chem.*, **130**, 722 (1939); D. Rittenberg and G. L. Foster, *ibid.*, **133**, 737 (1940). See also: D. Shemin and G. L. Foster, *Ann. N. Y. Acad. Sci.*, **47**, 119 (1946); M. D. Kamen, *Radioactive Tracers in Biology*, Academic Press, New York, 1947, pp. 112-118.

titative analysis now requires highly efficient and tedious fractionations, the method may be applied with little difficulty once a supply of compounds is available containing the suitable tracer isotopes.

Calculation of results is straightforward and depends on expressing the conservation of mass of the tracer. Let x be the unknown number of gram atoms of the tracer element in the compound originally present, and α be the natural concentration of the rare isotope in atom per cent. Let y gram atoms of the tracer element in the form of the same compound be added with a tracer isotope content of $(\alpha + C_0)$, where C_0 is the atom per cent *excess* of the tracer above the natural content. After mixing, any sample of the combination recovered may be analyzed and its isotope content expressed as $(\alpha + C)$, where C is the atom per cent *excess* of tracer in the final material. From the requirement that the number of atoms of tracer be conserved:

$$x\alpha + y(\alpha + C_0) = (x + y)(\alpha + C) \quad (14)$$

The solution of this for x gives:

$$x = y(C_0 - C)/C \quad (15)$$

Since the weight of compound, x' , originally present is desired rather than the gram atoms of element, x must be multiplied by M_1/n , where M_1 is the molecular weight of the unlabeled compound and n is the number of atoms of tracer element per molecule. For convenience, y , the gram atoms of carrier element added, may be similarly converted to y' , the weight of carrier compound, by the factor M_2/n , where M_2 is the molecular weight of the labeled compound. Combining:

$$x' = x(M_1/n) \quad \text{or} \quad x = x'(n/M_1) \quad (16)$$

and:

$$y' = y(M_2/n) \quad \text{or} \quad y = y'(n/M_2) \quad (17)$$

and substituting in equation (15) gives:

$$x' \frac{n}{M_1} = y' \frac{n}{M_2} \left(\frac{C_0 - C}{C} \right) \quad (18)$$

This leads to the final working equation:

$$x' = y' \left(\frac{C_0 - C}{C} \right) \frac{M_1}{M_2} \quad (19)$$

For most purposes, the addition of the labeling isotope changes the molecular weight of the compound so little that for practical purposes $M_1/M_2 = 1$

(e. g., if 32% N^{15} is used to label glycine, $M_1/M_2 = 0.996$), and equation (19) may be simplified to the more usual form:

$$x' = y'(C_0 - C)/C$$

The inverse of the ordinary isotope dilution technique is to determine the amount of a labeled substance formed in a reaction and present in a mixture of products, by adding a known amount of the natural, unlabeled compound and then recovering, by a process of fractionation, some of this constituent and determining the concentration of isotope. In a separate sample recovered without addition of unlabeled carrier, one determines the concentration of label present in the original material. When this cannot be done, the original concentration of label may be determined from a consideration of its source in the reaction, or by adding different quantities of carrier to two aliquots of the unknown, and setting up simultaneous equations which will yield both the amount of unknown product and its original isotope content.⁹⁰ A suggested use of the inverse method is in the analysis of reaction products obtained when enzymes or tissues slices are maintained in a labeled medium.

For inverse dilution calculations, C_0 becomes the excess concentration of tracer in the original x'' grams of compound, and C is the excess in the fraction recovered after adding y'' grams of unlabeled material. The equation analogous to (14) now becomes:

$$x(\alpha + C_0) + y\alpha = (x + y)(\alpha + C) \quad (20)$$

and the final working equation is:

$$x'' = y'' \left(\frac{C}{C_0 - C} \right) \frac{M_2}{M_1} \quad (21)$$

where M_2 and M_1 are, respectively, the molecular weights of labeled and unlabeled forms.

When the original substance to be determined in a mixture is unlabeled, it is sometimes possible to introduce the label by reaction with a labeled reagent to form quantitatively a labeled derivative which may then be treated as explained.⁹¹ Results yield the weight of derivative present, from which the weight of original compound can be calculated. This variation, like the inverse method in general, has the advantage that it is unnecessary to synthesize a large number of labeled compounds since one reagent may serve for a number of different determinations.

For a discussion of chemical considerations affecting the precision obtainable by the direct and inverse isotope dilution techniques, the reader is referred to the review by Radin.⁹⁰ Insofar as mass spectrometry is con-

⁹⁰ K. Bloch and H. S. Anker, *Science*, **107**, 228 (1948). N. S. Radin, *Nucleonics*, **1**, No. 2, 48 (1947).

⁹¹ A. S. Keston, S. Udenfriend, and R. K. Cannan, *J. Am. Chem. Soc.*, **68**, 1390 (1946); **71**, 249 (1949).

cerned, it appears that an over-all precision of about $\pm 0.5\%$ in the determination of most compounds is the best that can be done at present.

The method has also been applied to the direct elemental analysis of organic compounds for oxygen,⁹² carbon,⁹³ and nitrogen,⁹⁴ using O^{18} , C^{13} , and N^{15} . In general, a sample of the compound is burned or pyrolyzed at a high temperature with a measured quantity of a tracer compound such as O_2^{18} or $N^{15}H_3$ and equilibrium products are then examined for the degree of isotope dilution. Ideally, it should be possible to determine carbon, hydrogen, nitrogen, and oxygen simultaneously by high-temperature equilibration with a measured quantity of a universal reagent containing deuterium, C^{13} , N^{15} , and O^{18} and subsequently determining the four necessary isotope ratios. Difficulties with the analysis of deuterium, discussed on page 2031, have so far limited attempts to determine hydrogen in this way.⁹⁴

In the analysis of organic materials for nitrogen, it has been shown that, instead of using N^{15} , some other internal standard, such as neon, may be added to the unknown before carrying out the Dumas combustion. Without quantitatively recovering the gaseous products, a ratio determination of Ne:N by the mass spectrometer will then reveal the amount of nitrogen in the sample.⁹⁵

D. CHEMICAL PROCESSES INCIDENTAL TO THE TRACER TECHNIQUE

In all the uses which have been discussed, there are common chemical problems which involve the synthesis of labeled starting compounds and the conversion of product materials to a form suitable for mass spectrometer analysis. Stable tracer isotopes are generally available in the following chemical forms: deuterium as water or deuterium gas, carbon as potassium cyanide or barium carbonate, nitrogen as an ammonium salt, and oxygen as water or oxygen gas. From these, intermediates of general utility are often prepared commercially—as methyl iodide C^{13} , potassium phthalimide N^{15} , and nitric acid N^{15} —but syntheses must still be carried out for individual specific compounds.⁹⁶ In principle, these syntheses will be the same for both radioactive and stable tracers. A comprehensive list of synthetic compounds containing isotopes of carbon and nitrogen, to-

⁹² A. V. Grosse, S. G. Hindin, and A. D. Kirshenbaum, *J. Am. Chem. Soc.*, **68**, 2119 (1946); *Anal. Chem.*, **21**, 386 (1949).

⁹³ A. V. Grosse, A. D. Kirshenbaum, and S. G. Hindin, *Science*, **105**, 100 (1947).

⁹⁴ A. D. Kirshenbaum, S. G. Hindin, and A. V. Grosse, *Nature*, **160**, 187 (1947).

⁹⁵ S. G. Hindin and A. V. Grosse, *Anal. Chem.*, **20**, 1019 (1948).

⁹⁶ *Proceedings, Symposium on the Use of Isotopes in Biological Research*. University of Chicago, March, 1947. W. Sakami, W. E. Evans, and S. Gurin, *J. Am. Chem. Soc.*, **69**, 1110 (1947).

gether with references to published methods of preparation, has been given by Vennesland.⁹⁷

From the reaction products, the compound containing the tracer must be converted to a gas suitable for use in the spectrometer. Air, in general, must be excluded from the final sample because of its effect on the hot filament and because of its diluting effect if oxygen or nitrogen isotope ratios are to be determined. The conversion apparatus requires a vacuum system, the rest of the equipment depending upon the reaction which is to be carried out. A review of sample preparation methods in the mass spectrometric analysis of nitrogen, hydrogen, carbon, oxygen, and sulfur is available.^{97a}

When deuterium is to be analyzed, hydrogen gas must be prepared, usually by the chemical decomposition of water. If samples of water are large enough (a few milliliters), hydrogen gas for the analysis may be prepared by equilibrating tank hydrogen (or pure tank deuterium) with the water in a closed vessel over a platinum oxide catalyst. The deuterium content of the water may be calculated from the observed peaks at masses 2, 3, and 4, the known equilibrium constants for the exchange between H_2 , HD, and D_2 with H_2O , HDO, and D_2O , and from the known amount of hydrogen gas which was added.⁹⁸ When hydrogen is prepared by completely decomposing the water sample,⁹⁹ the mass spectrometric analysis of hydrogen is done by determining the ratio of mass 2 (H^1H^1) to mass 3 (H^2H^1) when only small amounts of H^2 are present, and using the ratio of mass 3 to mass 4 (H^2H^2) when the material is nearly pure deuterium.^{30, 100} Errors up to 50% are introduced by the formation of triatomic ions such as $(H^1H^1H^1)^+$. These are formed by secondary processes which are linearly dependent on the pressure in the ion source, and their presence may be corrected for by determining the mass 2:mass 3 ratio at several source pressures, and extrapolating to the true ratio at zero pressure.

The analysis of deuterium is also complicated by other effects not usually encountered with tracers. Since the percentage difference in mass between deuterium and hydrogen is so large, conditions must be changed radically to bring ions of mass 2 and mass 4 successively into focus at the collector slit, and this may introduce discrimination, so that the observed ratio of ions at these masses differs from the true ratio. Magnetic scanning

⁹⁷ B. Vennesland, in *Advances in Biological and Medical Physics*. Vol. I, J. H. Lawrence and J. G. Hamilton, eds., Academic Press, New York, 1948, Chapter II.

^{97a} D. B. Sprinson and D. Rittenberg, *U. S. Naval Med. Bull. Supplement*, Mar.-April, 1948, pp. 82-93.

⁹⁸ R. B. Fischer, R. A. Potter, and R. J. Voskuyl, *Anal. Chem.*, **20**, 571 (1948).

⁹⁹ F. C. Henriques and C. Margnetti, *Ind. Eng. Chem., Anal. Ed.*, **18**, 420 (1946).

¹⁰⁰ A. O. Nier, in *Preparation and Measurement of Isotopic Tracers*. Wilson, et al., eds., Edwards, Ann Arbor, 1946, pages 11-30.

is particularly desirable in this case. If an auxiliary source magnet is used, its effect on the ratio must also be checked since it will affect the trajectories of ions in the source. A serious memory effect is frequently found when analyzing hydrogen, due to adsorbed material in the instrument which contaminates successive samples. To minimize this effect, samples of widely different deuterium content should not be run in succession. Thorough flushing of the instrument with the next sample should be carried out before each analysis, and frequent bake-outs should be made.

Deuterium is the only stable tracer which may be analyzed conveniently by methods not requiring a mass spectrometer. Accurate determinations of density on highly purified water samples yield good results with either the falling-drop or the gradient-tube method,¹⁰¹ but the purity requirements are much higher than when hydrogen gas is to be analyzed in the spectrometer. Effects due to O^{18} are also lumped with deuterium. Techniques for the determination of density are described by Bauer in Chapter VI.

In work with N^{15} , nitrogen gas is usually prepared for analysis by treating an ammonium salt (as from a Kjeldahl digestion) with alkaline hypobromite solution. Both reactants must be free from air before mixing and must be combined under vacuum.¹⁰² Nitrogen evolved is transferred to a sample bulb by a Toepler pump. Oxygen for analysis is usually converted to CO_2 or, if in the form of water to start with, is shaken with a known quantity of ordinary CO_2 , which then assumes the equilibrium concentration of O^{18} : O^{16} characteristic of the whole system.¹⁰³ The water can then be frozen and the CO_2 pumped off for analysis. Samples of C^{13} are generally analyzed in the form of CO_2 , obtained as the product of a combustion or a wet oxidation. The CO_2 is frozen out with liquid air to permit the removal of excess oxygen by pumping, and is then transferred to a sample tube.¹⁰⁴ The CO_2 should be dry, and the presence of hydrogen in the sample should also be avoided, since the dissociation of either H_2 or H_2O can lead to secondary reactions and the production of HCO_2^+ ions.¹⁰⁵ This effect will be a function of the pressure in the ion source, and may lead to appreciable errors in the determination of C^{13} from a measurement of the mass 45:mass 44 ratio in carbon dioxide. Stable sulfur is usually analyzed as SO_2 .

¹⁰¹ M. Cohn, in *Preparation and Measurement of Isotopic Tracers*, Wilson, et al., eds., Edwards, Ann Arbor, 1946, pages 51-59. C. Anfinson, *ibid.*, pages 61-65.

¹⁰² D. Rittenberg, *ibid.*, pages 31-42. See also D. B. Sprinson and D. Rittenberg, *J. Biol. Chem.*, **180**, 711 (1949).

¹⁰³ M. Cohn and H. C. Urey, *J. Am. Chem. Soc.*, **60**, 679 (1938).

¹⁰⁴ S. Weinhouse, in *Preparation and Measurement of Isotopic Tracers*, Wilson, et al., eds., Edwards, Ann Arbor, 1946, pages 43-47.

¹⁰⁵ J. J. Mitchell, R. H. Perkins, and F. F. Coleman, *J. Chem. Phys.*, **16**, 835 (1948).

E. LIMITATIONS AND ACCURACY OF THE METHOD

The fundamental limitation on the use of stable tracer atoms is the amount by which they may be diluted with normal material and still be measured with the required accuracy. This question has been discussed in Chapter XXX and shown, for both radioactive and stable tracers, to depend on: (1) the initial concentration of the tracer available, (2) the minimum concentration in excess of normal which can be detected, and (3) the maximum permissible uncertainty in the result. An additional factor will be introduced by any variation which occurs in the supposedly "constant" isotopic composition of "normal" material. Usually such variation will be of the same order of magnitude as the uncertainty in the mass spectrometer determination.

With the best techniques, Nier, Ney, and Inghram⁷⁴ have shown that the $C^{13}:C^{12}$ ratio in CO_2 (near the normal value) can be determined with an accuracy better than 0.1%. In another series of determinations, Nier²⁹ has shown that analyses of C^{13} in fourteen near-normal samples of CO_2 could be repeated after two days with a mean deviation of 0.3% in the ratio. In this particular series, all fourteen of the analyses were slightly higher on the second determination, indicating some systematic change in the apparatus. In an evaluation of the over-all accuracy possible with the spectrometer, Nier¹⁰⁰ states that a 0.5% change in the abundance ratio is probably the minimum which can be detected in most cases, although 0.25% is not out of reason if sufficient care is taken. This means that C^{13} will be "lost" when diluted so that its ratio to C^{12} is below 0.01105 (assuming normal to be 0.01100). Starting with pure C^{13} , this would allow an ultimate dilution of 20,000 times, and the final concentration would have an uncertainty of 100% (*i. e.*, $0.005 \pm 0.005\%$ C^{13} in excess of normal). If 10% accuracy is required, a dilution of only 2000 could be tolerated, and if only 50% C^{13} were initially available, there would be a further reduction to a maximum 1000-fold dilution. While these dilutions do not match those available with radioactive tracers, it has been found that much work in biochemistry and essentially all that may be contemplated in organic chemistry, need not approach the limits established for stable tracers.

F. CALCULATION OF RESULTS

Assuming that the mass spectrometer has given values for the various ion abundances, a final problem is to express the result in terms of the mole per cent of the tracer present. This process may be illustrated by a typical calculation using N^{15} .

The analysis is made on N_2 , and the spectrometer reveals peaks due to $N^{14}N^{14}$, $N^{14}N^{15}$, and $N^{15}N^{15}$. Let n = atom per cent N^{15} and $(100 - n)$ = atom per cent

N^{14} , then the abundance of the mass 28 species will be proportional to $(100 - n)^2$, the abundance of mass 29 to the quantity $2n(100 - n)$, and that of mass 30 to n^2 . If the ratio, (mass 28)/(mass 29), is measured and found equal to R , then:

$$R = (100 - n)^2/2n(100 - n) \quad (22)$$

and:

$$n = 100/(2R + 1) \quad (23)$$

Similarly, if the observed ratio, (mass 29)/(mass 30) = R' , then:

$$n = 200/(2 + R') \quad (24)$$

It is often desirable to measure both the (28)/(29) and the (29)/(30) ratios and calculate the per cent of N^{15} independently from each. This is a check on the extent of equilibrium attained among the ion species (which should be complete), and on the purity of the sample. A contamination with atmospheric nitrogen or with any impurity of mass 28, 29, or 30 is thus revealed.

In the mass analysis of substances like CO_2 when the per cent of C^{13} is desired, it is necessary to take into account isotopic variations in the other atoms of the molecule. In CO_2 , the peaks at mass 44 ($C^{12}O^{16}O^{16}$) and mass 45 ($C^{13}O^{16}O^{16}$) are compared, but oxygen of mass 17 can also produce an ion of mass 45 ($C^{12}O^{16}O^{17}$). The natural abundance of O^{17} is only 0.04%, so this correction is negligible in most cases. If very small increases in the C^{13} concentration are to be measured, a reduction in the observed peak height at mass 45 must be made to the extent of 0.04% of the observed peak at mass 44 for each of the oxygen atoms in the molecule; the total correction is thus 0.08% of the peak at mass 44.

2. Determination of Ionization Potentials and Strength of Chemical Bonds

A. MECHANISMS OF IONIZATION AND DISSOCIATION BY ELECTRON IMPACT

When a molecule is subjected to electron bombardment, a wide variety of ionization and dissociation processes may take place. Except for the simplest molecules, no theory has been developed which permits calculation of the various reaction probabilities or of the effect of increasing either the energy of the electrons or the temperature of the gas molecules.

If electrons of low energy are employed, such as are obtained when only a few volts are applied between the filament and the ionizing case, the energy transmitted to the molecule upon impact serves only to excite it to higher rotational, vibrational, or electronic energy levels; these cannot be detected by the mass spectrometer. Upon increasing the electron-accelerating voltage, a point is reached at which enough energy is available to eject an electron from the molecule, forming a positive ion which can be detected. The particular energy characterizing the bombarding electrons

at this point equals the ionization potential of the molecule. It must be noted that the e. m. f. accelerating the electrons may not correspond to their true energy because of the spread in velocity as the electrons leave the hot filament. To correct for this and for effects such as space charge, a gas, for which the true ionization potential is accurately known from spectroscopic data, is measured under similar conditions and a voltage correction is determined which can be applied to similar molecules of unknown ionization potential. The method is subject to from five to ten times the error of direct measurements from band spectra,¹⁰⁶ but it is of value when the results can be obtained only in this way. The principal errors, aside from the voltage correction, lie in detecting the ion beam when it is first formed at near-zero intensity.¹⁰⁷ The significance of two common methods for doing this is discussed by Mariner and Bleakney, and an alternate method, which is claimed to give reproducibility within ± 0.02 v., is described by Honig.¹⁰⁸ A valuable review of electron impact methods, as applied to diatomic molecules, is also available.¹⁰⁹

B. SIGNIFICANCE OF APPEARANCE POTENTIALS

If slightly more energy is given to the electrons, the excitation to the ionized state may be at a point on the ionic potential energy curve above the dissociation limit. The excited ion will then undergo dissociation, with the breaking of a chemical bond, so that ions of lower weight will appear in the mass spectrum, and neutral fragments may also be formed. The lowest electron energy at which a new ion is formed is termed its appearance potential, and is equal to the sum of the following quantities: (1) the energy of breaking the chemical bond, (2) the ionization potential of the fragment ionized, and (3) the kinetic or excitational energy of all of the fragments. The third term has been found to be small (< 0.5 e. v.) for many processes.

In a complex molecule, it is seldom possible to predict which bond will be broken most easily, and as electron energies increase above the first appearance potential, other dissociation reactions set in, each requiring a different energy of excitation. Smith,¹¹⁰ in studying the ions formed in methane, has observed more than a dozen reactions of the type listed below, and has measured the appearance potentials of the positive ions formed:

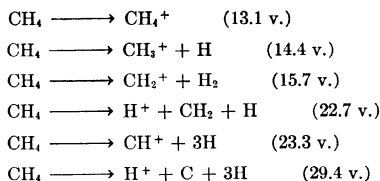
¹⁰⁶ T. Mariner and W. Bleakney, *Phys. Rev.*, **72**, 792 (1947).

¹⁰⁷ J. J. Mitchell and F. F. Coleman, *J. Chem. Phys.*, **17**, 44 (1949).

¹⁰⁸ T. Mariner and W. Bleakney, *Phys. Rev.*, **72**, 807 (1947). R. E. Honig, *J. Chem. Phys.*, **16**, 105 (1948).

¹⁰⁹ A. G. Gaydon, *Dissociation Energies and Spectra of Diatomic Molecules*, Chapman & Hall, London, 1947, Chapter VII, pages 126-138.

¹¹⁰ L. G. Smith, *Phys. Rev.*, **51**, 263 (1937).



In this system, negative ions corresponding to CH_2^- , CH^- , C^- , and H^- were also observed when the ion-accelerating potential was reversed.

Appearance potential studies on more than forty molecules have already been reported and the dissociation spectra of many others at particular ionizing voltages have been studied for the purpose of gas analysis, to be discussed in the next section. Smyth¹¹¹ has reviewed the appearance potential data to 1931 and Hipple³¹ has extended the review to 1942. A recent comprehensive study of the ionization and dissociation of paraffin hydrocarbons by electron impact¹¹² is typical of recent work. A study of the H_2 and D_2 molecules, for which the theory is most complete, is also of interest.¹¹³

In order to be of greatest use, measurements must be combined with thermochemical and spectroscopic data in order to express the appearance potentials in terms of the ionization potentials and the bond strengths (plus any kinetic or excitational energies) which contribute to the observed effects.

When the mass spectrum of a molecule has once been obtained under given ionizing conditions, it is interesting to inquire what changes, if any, result from the introduction of isotopes into the compound. Deuterium has been used to replace hydrogens in methane, ethane, and propane and the resulting mass spectra have been compared with those of the usual hydrocarbons.¹¹⁴ It is found that, not only does a C—D bond have a probability of breaking under electron bombardment only about one half as great as does a C—H bond (an effect which might be predicted from the effect of mass on zero-point energies and on activation energies), but the presence of a C—D bond in the molecule weakens neighboring C—H bonds, so that the mass spectrum is significantly altered.

The effect on the mass spectrum of substituting C^{13} for C^{12} in propane

¹¹¹ H. D. Smyth, *Revs. Modern Phys.*, **3**, 347 (1931).

¹¹² M. B. Koffel and R. A. Lad, *J. Chem. Phys.*, **16**, 420 (1948).

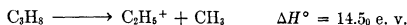
¹¹³ D. P. Stevenson, *J. Chem. Phys.*, **15**, 409 (1947). N. Bauer and J. Y. Beach, *ibid.*, **15**, 150 (1947).

¹¹⁴ J. Turkevich, L. Friedman, E. Solomon, and F. M. Wrightson, *J. Am. Chem. Soc.*, **70**, 2638 (1948). J. Delfosse and J. A. Hipple, *Phys. Rev.*, **54**, 1060 (1938). M. Evans, N. Bauer, and J. Y. Beach, *J. Chem. Phys.*, **14**, 701 (1946).

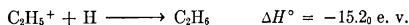
has also been investigated.¹¹⁵ Here, the effect was surprisingly large, considering the relatively small mass difference between the two isotopes, with the probability of breaking a $C^{12}-C^{12}$ bond about twenty per cent greater than for a $C^{12}-C^{13}$ bond.

C. CALCULATION OF BOND STRENGTHS

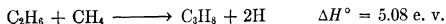
As an example of the application of appearance potentials to the calculation of bond strengths, the work of Stevenson¹¹⁶ may be cited. When propane is bombarded with electrons, the appearance potential of $C_2H_5^+$ is 14.5₀ v., or:



The energy put into this reaction is assumed to equal the sum of $D(CH_3-C_2H_5) + I(C_2H_5)$, where D is the strength of the bond indicated and I is the ionization potential of the fragment. Similarly, when ethane is dissociated, the appearance potential of $C_2H_5^+$ is 15.2₀ v., or (writing the equation in the reverse direction):



The appearance potential equals $D(C_2H_5-H) + I(C_2H_5)$. From thermochemical data obtained separately:



If these three equations are combined by addition, it will be noticed that the value of $I(C_2H_5)$ disappears from the difference of the two appearance potentials, and:



This value of the C—H bond strength has an uncertainty of about ± 0.2 e. v., and corresponds to a value of 101 ± 4.5 kcal. per mole. Stevenson points out that this is in good agreement with the value of 103 kcal. per mole calculated theoretically, and about equals the mean of experimental determinations by kinetic and by photochemical methods, which yield 108 kcal. per mole and 94.8 kcal. per mole, respectively.

3. Direct Gas Analysis

A. PRINCIPLES

In tracer work, the mass spectrometer is used to measure the abundance ratios of isotopic molecules existing in a mixture. The extension of the

¹¹⁵ O. Beeck, J. W. Otvos, D. P. Stevenson, and C. D. Wagner, *J. Chem. Phys.*, **16**, 255 (1948).

¹¹⁶ D. P. Stevenson, *J. Chem. Phys.*, **10**, 291 (1942).

method to the analysis of various dissimilar molecules in a mixture has been one of the most fruitful developments in mass spectrometry. One of the earliest applications of the method was the examination of minute samples of soil gases from regions being prospected for petroleum. Too small for analysis by chemical methods, these samples, when admitted to a mass spectrometer, would reveal the presence of ethane and heavier hydrocarbons, indicating the subsurface presence of oil or natural gas. Since 1940, the use of the spectrometer in direct gas analysis has been extended so that complex mixtures of as many as 15 or 20 different components are now analyzed routinely with a speed and precision that exceed the best available chemical methods.

If ionizing conditions in the mass spectrometer, including the number and energy of the ionizing electrons, the temperature of the ion source, and the ion-accelerating voltages, are all held constant and a pure gas is admitted, it will produce an ionization and dissociation spectrum typical of the molecule. Methane, as previously shown, will produce all of the positive ions from CH_4^+ down through the series CH_3^+ , CH_2^+ , CH^+ , to C^+ and H^+ if the electrons have an energy greater than about 30 volts.

In addition, doubly and even triply charged ions are obtained from most molecules if sufficient energy is available. Since the spectrometer actually measures the ratio m/e , a doubly charged ion of mass 60 will be collected with singly charged ions of mass 30, and a fragment of mass 61, if doubly ionized, will give an apparent peak at mass 30.5 on the m/e scale. A detailed study of doubly charged ions in the spectra of hydrocarbons has been reported,^{116a} but the peaks are generally too small to be useful. Negative ions are also usually disregarded since they are not accelerated out of the ionization chamber by the negative voltage gradient.

In most analytical work, electron energies are set high enough to form all possible ions—usually at 75 volts or above. At this voltage, although a more complex spectrum is produced, small additional changes in electron energy produce negligibly small effects, so that stabilization of the dissociation process is somewhat easier. The relative abundance of each possible ion species will, in general, be unpredictable in going from compound to compound, but as long as the ionizing conditions remain constant and the number of molecules being ionized is large enough for the probability laws to apply, the pattern of ions will be typical and reproducible for each substance, with the peak heights appearing in a constant ratio to each other.

Assuming that the pure gas is being admitted to the system through a

^{116a} F. L. Mohler, *et al.*, *J. Research Natl. Bur. Standards*, **42**, 369 (1949).

¹¹⁷ A. K. Brewer and V. H. Dibeler, *J. Research Natl. Bur. Standards*, **35**, 125 (1945).

capillary leak or orifice, and that pumping rates and other flow conditions remain constant, the intensity of each mass peak in the spectrum of a particular compound is proportional to the pressure of the gas in the ion source (since this determines the frequency with which ionizing collisions occur), and this in turn is proportional to the amount of gas flowing through the leak, and so to the pressure behind the leak. If the sample pressure is once measured accurately and the intensities of the various ion beams are determined at the same time, then future readings of any single ion-beam intensity can be interpreted in terms of pressure of the pure sample.

As described on page 2008, the quantity of gas flowing through the leak may also be expressed in terms of the weight of sample admitted to the inlet system from a calibrated micropipette. This eliminates the need to read the sample pressure on a manometer before expansion, and results may be calculated directly on a weight basis, instead of in terms of mole fractions derived from partial pressures.

In order to analyze mixtures of different gases, the same requirement of proportionality between sample pressure and ion peak heights must hold for each of the components separately. For this to occur, each gas must flow through the leak independently of any other gas present, and at a rate determined purely by its own pressure in the mixture. As shown on page 2010, this is characteristic of molecular flow and can be provided by a suitable pressure in the sample chamber and a sufficiently small leak. In addition, each gas in the ionization chamber must yield ions in numbers independent of the pressure of other substances. This will be accomplished when ion source pressures are low enough so that only a negligible number of secondary collisions remove ions which otherwise would be accelerated. Finally, ions formed in the source from one substance must reach the collector without interference from ions or molecules of the other components. This requires a low pressure in the analyzer region as well as in the source. All of these requirements may be summarized by saying that the mass spectrum obtained from a mixture must equal that which would be obtained if the components were run separately at their respective partial pressures and the peak heights at each value of m/e added, to form a composite spectrum. The importance of this principle of "linear superposition" has been emphasized by Washburn and others.^{42, 43, 113}

A second principle, of equal practical importance, is that the design of the spectrometer shall allow as little drift as possible in conditions which affect the cracking pattern of pure gases. Since these patterns must be determined for calibrating purposes, changes or drifts greatly increase the

¹¹³ H. W. Washburn, H. F. Wiley, S. M. Rock, and C. E. Berry, *Ind. Eng. Chem., Anal. Ed.*, 17, 74 (1945).

number of standards which must be run for each unknown mixture and decrease the accuracy which is obtained.

Although the magnitude of the effect differs widely from compound to compound, it is found that the temperature of the gas is a critical variable. Figure 10 is reproduced from the work of Fox and Hipple¹¹⁹ who introduced a mixture of isobutane and neon into an ion source through a metal block whose temperature could be measured and controlled. Some of the ions obtained from isobutane included mass 58 ($C_4H_{10}^+$), mass 57 ($C_4H_9^+$), mass 43 ($C_3H_7^+$), and mass 41 ($C_3H_5^+$), and the intensities of these relative to

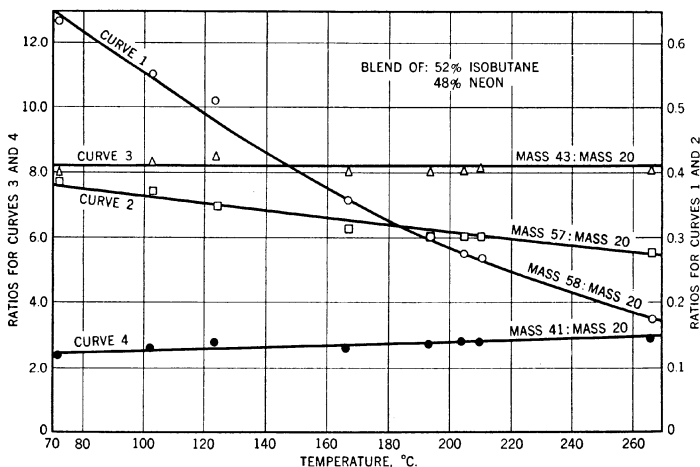


Fig. 10.—Variation with temperature of ion abundance ratios in isobutane-neon mixture,¹¹⁹

the Ne^+ peak at mass 20 were recorded. The curves showed a strong negative temperature coefficient for the parent ion, with almost no temperature effect on mass 43, and a slight positive effect on mass 41. Other hydrocarbons were found to show even larger effects. The branched hydrocarbon 2,2,3-trimethylpentane formed more than five times more ions of the parent mass at 175° C. than at 225° C. This indicates that source temperatures must be controlled within $\pm 1^\circ$ C. if cracking patterns are to be reproducible. The best source temperature for gas analysis with the particular instrument used by Fox and Hipple was above 200° C.

Other undesirable factors affecting cracking patterns over a period of

¹¹⁹ R. E. Fox and J. A. Hipple, *J. Chem. Phys.*, **15**, 208 (1947). For a general explanation of the effect, see D. P. Stevenson, *J. Chem. Phys.*, **17**, 101 (1949).

time include: (1) the formation of nonconducting deposits on various accelerating plates which cause a gradual distortion of electrostatic fields, (2) movement of the filament due to expansion and contraction or changed alignment when a new filament is installed, (3) changes in discrimination between ions in the source, for reasons already discussed, and (4) changes in pumping conditions, sometimes caused by the trapping of particularly volatile components from previous samples in the cold trap of the vacuum system. Other factors no doubt exist, for even with the most stable instruments, and when analyzing routine samples, the operator must usually devote about 20% of potential operating time to calibrations, sensitivity checks, and adjustments, in addition to about two days per month for repairs and more extensive calibrations.¹²⁰ Washburn³⁹ states that, under favorable conditions, spectra will stay sufficiently constant for a period of one or two weeks. The critical components, however, will require more frequent calibration, as will samples of a nonroutine nature.

B. ANALYSIS OF SIMPLE MIXTURES

When the components of a mixture are simple molecules not producing a complex spectra, and particularly when there is no overlapping of the peaks, analysis is a rapid and simple matter. Each component is run separately at a measured sample pressure (or sample weight), and for each a sensitivity factor is determined giving the units of peak height observed per unit pressure (or weight) in the sample for one typical value of m/e . The particular peak chosen must not appear in the spectra of the other components, and should be one of the larger peaks in the spectrum in order to obtain maximum sensitivity. When the unknown is run, the ion current measured for each of these peaks is divided by the corresponding sensitivity factor to give the pressure (or weight) of each component in the mixture. The sum of such calculated pressures should equal the observed total pressure of the unknown. If it does not, the presence of an unsuspected component is indicated, or if this cannot be confirmed by locating peak ratios foreign to the known components, an error in calibration, measurement, or calculation may be suspected. When a weight calibration is used from a graduated micropipette, the density of each unknown liquid mixture must be measured if it is desired to check the sum of the individual component weights against the total for the sample.

Applications of this type are found in the analysis of rare gas mixtures, the measurement of nitrogen in helium, or the measurement of traces of oxygen in nitrogen atmospheres of annealing furnaces.³¹ In the latter application, measurements were made on concentrations down to 0.001% of O₂.

¹²⁰ W. S. Young, *Natl. Petroleum News*, **38**, R212 (1946).

C. CRACKING PATTERNS OF PURE ORGANIC SUBSTANCES

Although organic compounds containing oxygen, sulfur, and halogens, in addition to carbon and hydrogen, have been studied and quantitatively determined in the mass spectrometer, by far the greatest amount of work has been done with hydrocarbons. Cracking patterns for some 150 different compounds had been determined up to 1946¹²¹ and at that time 79 different standard samples of hydrocarbons were available from the National Bureau of Standards for mass spectrometer calibration purposes. Since then, many additional spectra have been obtained. Included now are many of the paraffin hydrocarbons through the C₉ series, olefins through most of the pentenes, many diolefins, naphthenes from C₆ to C₇, aromatics from C₆ to C₁₀, and acetylenes from C₂ to C₄. The most complete atlas of mass spectra available is that being published serially by the Bureau of Standards in cooperation with the American Petroleum Institute.¹²² Spectra are compiled by users of various types of mass spectrometers and measurements of all significant peaks are expressed as percentages of the principal peak for each compound. Absolute sensitivities per micron of sample pressure behind the leak are given, as well as comparable sensitivity data for *n*-butane which may be used as a basis of comparison between different instruments.

From the simplest hydrocarbon molecules, methane and ethane, the most abundant ions formed are those of the undissociated parent molecules. With increasing numbers of carbon atoms, peaks of lower mass than the parent—representing the breaking of one or more bonds, either C—C or C—H—become more abundant until, in highly branched higher hydrocarbons, ions corresponding to the simple loss of an electron from the parent molecules are not observed. Ions formed by breaking one of the bonds around a quaternary carbon are usually most abundant, and tertiary bonds also appear more easily broken than do primary or secondary C—C linkages.

The behavior of isomeric molecules under electron impact is of particular interest since the analysis of mixtures of isomers is impossible unless the spectrum obtained from each is characteristic of the configuration. As an example of the information available, the work of Stevenson and Hipple¹²³ on *n*-butane and isobutane (both of mass 58) may be cited. Graphs of parts of the two spectra are shown in figure 11 where the height of each ion peak is a measure of its relative abundance. It will be noted, for example, that the abundance ratio of mass 58 to mass 57 is much greater for

¹²¹ A. K. Brewer, *ASTM Bull.*, No. 140, 38 (1946).

¹²² *Catalog of Mass Spectral Data*, American Petroleum Institute Research Project No. 44, National Bureau of Standards, Washington, D. C.

¹²³ D. P. Stevenson and J. A. Hipple, *J. Am. Chem. Soc.*, **64**, 1588 (1942).

the *n*-butane than for isobutane. This suggests that the latter compound undergoes fragmentation during ionization to a greater extent than does the straight-chain compound, thus reducing the probability of forming the undissociated $\text{iso-C}_4\text{H}_{10}^+$ ion. Because of this difference, which is carried into most of the other peak ratios as well, the isomers may be treated as

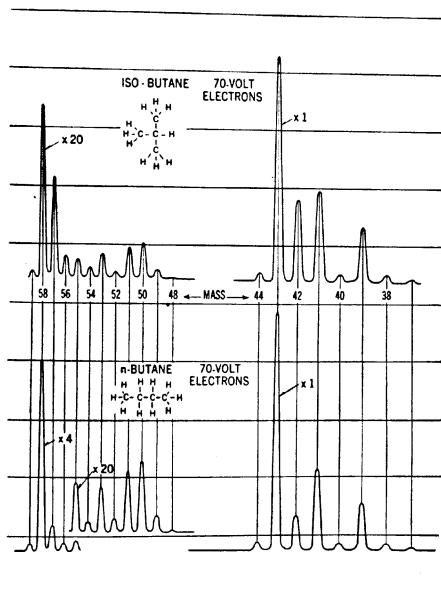


Fig. 11.—Portions of the mass spectra of isobutane (upper) and *n*-butane (lower).³¹

entirely independent compounds with overlapping spectra. The ionization and dissociation of *cis* and *trans* isomers by electron impact has also been studied with the mass spectrometer.¹²⁴

With isomers, it will be noted that fragments of certain masses are readily formed from one isomer by the rupture of a single bond while this is impossible from the other. Thus, from isobutane it is impossible to obtain a C_2 fragment by breaking any single C—C bond. If ions such as C_2H_5^+ are observed at all, it must be as a result of a molecular rearrangement taking place at the moment of dissociation, and this requirement usually reduces

¹²⁴ V. H. Dibeler, *J. Research Natl. Bur. Standards*, **38**, 329 (1947).

the probability of forming the particular ion. In *n*-butane, breaking the central C—C bond will yield two C_2 fragments, and the cracking patterns of the two butanes¹¹⁷ confirm the expected difference in the mass 29 peaks. Similarly, Washburn³⁹ points out, among other examples, that 3-methylheptane can form C_4 fragments directly while 4-methylheptane cannot do so without rearrangement or the simultaneous breaking of two C—C bonds. In this case, the $C_4H_9^+$ peak from the 3-methyl isomer is four times as large as from the 4-methylheptane.

One of the most complete studies of the mass spectra of isomers has been carried out on the octanes.¹²⁵ All eighteen isomers have been examined and certain relations between structure and dissociation pattern have been observed, but the authors conclude: (1) that correlation between structure and spectrum is still far from complete; and (2) that rearrangement of atoms to give "forbidden" ions is probable enough to require consideration, although the process is not at all understood.

Closely related to the problem of identifying isomers is that of locating the position of a tracer isotope which has been introduced into a molecule. If, for example, either deuterium or C^{13} is used to label propane, the mass spectrometer can not only determine the concentration of tracer (from H_2O or CO_2 prepared by combustion), but can also show, on the propane itself, whether the label is in the 1- or 2-position.^{114, 39} Thus, the CH_2D^+ ion (mass 16) occurs as a direct product of dissociation from $CH_3CH_2CH_2D$, but only as a product of rearrangement from CH_3CHDCH_3 .

D. RESOLUTION OF MIXTURE SPECTRA

When the mass spectrum of a complex mixture has been obtained under conditions which insure the additivity of contributions from individual components, its resolution into the individual spectra, and from these, the analysis of the mixture, is directly possible. In general terms, if a mixture of *n* components is analyzed, and a peak height, I_1 , is found at mass m_1 , we may write the linear equation:

$$S_{11}x_1 + S_{12}x_2 + \dots + S_{1n}x_n = I_1 \quad (25)$$

where S_{11} is the peak height at mass m_1 due to unit pressure of component 1 as determined in a pure sample, and x_1 is the partial pressure of component 1 in the unknown mixture. Similarly, S_{1n} is the peak height at mass m_1 due to unit pressure of component *n*, and x_n is the corresponding partial pressure of the *n*th component. In this equation, the values of *x* are unknowns and the values of *S* are constants determined from a calibration with the *n* components singly, each corrected to unit pressure.

¹²⁵ E. G. Bloom, F. L. Mohler, J. H. Lengel, and C. E. Wise, *J. Research Natl. Bur. Standards*, **41**, 129 (1948).

